

ESSENTIALS  
OF  
CHEMICAL PHYSIOLOGY



HALLIBURTON



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*A. S. Halliburton*

## CHEMICAL PHYSIOLOGY

*By the same Author.*

A TEXT-BOOK OF CHEMICAL PHYSIOLOGY  
AND PATHOLOGY.

With 104 Illustrations. 8vo. 28s.

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THE ESSENTIALS  
OF  
CHEMICAL PHYSIOLOGY

FOR THE USE OF STUDENTS

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1893

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## P R E F A C E

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THIS book is written with the object of supplying the student with directions for examining practically the most important of the subjects included under the heading Chemical Physiology, or Physiological Chemistry. At the same time it is intended to serve as an elementary text-book of the subject.

The plan of the work is the same as that adopted by Professor SCHÄFER, in his ‘Essentials of Histology.’ The practical lessons are followed by a brief description of the matter of which they treat. Each of the elementary lessons may be supposed to occupy a class for one hour. The advanced lessons will take more time—usually two hours.

The practical lessons are those which I have been using for many years past in my own classes. Their original source is the ‘Syllabus of Lectures’ published by Professor J. BURDON SANDERSON in 1879, and although some few of the lessons are but little altered, the greater number have been very considerably modified to include the results of recent researches.

The illustrations have for the most part been transferred from my text-book of ‘Chemical Physiology and Pathology.’ A few additional figures have been expressly drawn for this work. Figs. 4, 10, and 15 have been taken from YEO’s ‘Physiology’; figs. 16 and 17 from MCKENDRICK’s ‘Physiology’; figs. 51 and 52 from BRYANT’S ‘Surgery’; and figs. 73 and 74 from WALLER’s ‘Physiology.’ For leave to use

these I beg to tender my thanks to the respective authors and publishers.

The duty of reading the proof-sheets has been greatly lightened for me by my friend Mr. T. G. BRODIE, M.B., Demonstrator of Physiology in King's College, and I have to thank him for many valuable suggestions and alterations.

W. D. HALLIBURTON.

KING'S COLLEGE: *July* 1893.

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# ESSENTIALS OF CHEMICAL PHYSIOLOGY

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## INTRODUCTION

Chemical Physiology, or Physiological Chemistry, as it is sometimes termed, deals with the chemical composition of the body, and also of the food which enters, and the excretions which leave, the body.

When a chemist examines living things he is placed at a disadvantage when compared with an anatomist ; for the latter can with the microscope examine cells, organisms, and structures in the living condition. The chemist, on the other hand, cannot at present state anything positive about the chemical structure of living matter, as the reagents he uses will destroy the life of the tissue he is examining. There is, however, no such disadvantage when he examines non-living matter, like food and urine, and it is therefore in the analysis of such substances that chemical physiology has made its most important advances, and the knowledge so obtained is of the greatest practical interest to the student and practitioner of medicine.

The animal organism is in its earliest embryonic state a single cell ; as development progresses it becomes an adherent mass of simple cells. In the later stages various tissues become differentiated from each other by the cells becoming grouped in different ways, by alterations in the shape of the cells, by deposition of intercellular matter between the cells, and by chemical changes in the living matter of the cells themselves. Thus in some situations the cells are grouped into the various epithelial linings ; in others the cells become elongated, and form muscular fibres ; in the connective tissues we have a preponderating amount of intercellular material, which may become permeated with fibres, or be the seat of the deposition of calcareous salts, as in bone. Instances of chemical changes in the cells them-

selves are seen on the surface of the body, where the superficial layers of the epidermis become horny (*i.e.* filled with the chemical substance called keratin) ; in the mucous salivary glands, where the cells become filled with mucin, which they subsequently extrude ; and in adipose tissue, where they become filled with fat.

In spite of these changes, the variety of which produces the great complexity of the adult organism, there are many cells which still retain their primitive structure ; notable among these are the white corpuscles of the blood.

A cell may be defined as a mass of living material containing in its interior a more solid structure called the nucleus. The nucleus exercises a controlling influence over the nutrition and subdivision of the cell.

Living material is called protoplasm, and protoplasm is characterised by its (1) power of movement (seen in amœboid movement, ciliary movement, muscular movement) ; (2) its power of assimilation, that is, it is able to take in nutrient material or food, and convert it into protoplasm ; (3) its power of growth ; this is a natural consequence of its power of assimilation ; (4) its power of reproduction ; this is a variety of growth ; and (5) its power to excrete, to give out waste materials, the products of its other activities.

The chemical structure of protoplasm can only be investigated after the protoplasm has been killed. The substances it yields are (1) **Water** ; protoplasm is semifluid, and at least three-quarters of its weight, often more, are due to water. (2) **Proteids**. These are the most constant and abundant of the solids. A proteid or albuminous substance consists of carbon, hydrogen, nitrogen, oxygen, with sulphur and phosphorus in small quantities only. In nuclein, a proteid-like substance formed in the nuclei of cells, phosphorus is more abundant. White of egg is a familiar instance of an albuminous substance or proteid, and the fact (which is also familiar) that this sets on boiling into a solid will serve as a reminder that the greater number of the proteids found in nature have a similar tendency to coagulate under the influence of heat and other agencies. (3) Various other substances occur in smaller proportions, the most constant of which are **lecithin**, a phosphorised fat ; **cholesterin**, a monatomic alcohol ; and **inorganic salts**, especially phosphates and chlorides of calcium, sodium, and potassium.

It will be seen from this rapid survey of the composition of the body how many are the substances which it is necessary we should study ; the food from which it is built up is also complex, for animals do not possess to such an extent as plants do the power of building

up complex from simple materials. We may now proceed to an enumeration of the chemical constituents of the animal body, and group them in a systematic way.

The substances out of which the body is built consist of chemical elements and of chemical compounds, or unions of these elements.

The elements found in the body are carbon, hydrogen, nitrogen, oxygen, sulphur, phosphorus, fluorine, chlorine, silicon, sodium, potassium, calcium, magnesium, lithium, iron, and occasionally manganese, copper, and lead.

Of these very few occur in the free state. Oxygen (to a small extent) and nitrogen are found dissolved in the blood; hydrogen is formed by putrefaction in the alimentary canal; particles of carbon breathed in with the air may be found in the tissues of the lungs. With some few exceptions such as these, the elements enumerated above are found combined with one another to form what are called compounds.

The **compounds**, or, as they are generally termed in physiology, the *proximate principles*, found in the body are divided into—

- (1) Mineral or inorganic compounds.
- (2) Organic compounds, or compounds of carbon.

The **inorganic compounds** present are water, various acids (such as hydrochloric acid in the gastric juice), ammonia (as in the urine), and numerous salts, such as calcium phosphate in bone, sodium chloride in blood and urine, and many others.

The **organic compounds** are more numerous: they may be subdivided into—

1. Various groups of alcohols and organic acids, and their compounds, such as the fats and carbohydrates.
2. Various derivatives of ammonia, amides, amines, urea, &c.
3. Aromatic bodies, or derivatives of benzene.
4. Proteids, the most important of all, and substances allied to proteids like the albuminoids, pigments, and ferments.

If we study the chemical composition of foods we find that they also may be subdivided by chemical analysis into proximate principles, and these we may conveniently group as follows:—

Inorganic . . . . .	Nitrogenous	Water.
		Salts—e.g. chlorides and phosphates of sodium and calcium.
Organic	Non-nitrogenous	Proteids—e.g. albumin, myosin, casein.
		Albuminoids—e.g. gelatin, chondrin, nuclein.
	Non-nitrogenous	Simpler nitrogenous bodies—e.g. lecithin, creatine.
		Fats—e.g. butter, fats of adipose tissue.
		Carbohydrates—e.g. sugar, starch.
		Simple organic bodies—e.g. alcohol, vegetable acids, and salts.

Many of the substances enumerated above only occur in small quantities. The most important are the inorganic substances, *water* and *salts*; and the organic substances, *proteids*, *carbohydrates*, and *fats*. It is necessary in our subsequent study of the principles of chemical physiology that we should always keep in mind this simple classification; the subdivision of organic substances into proteids, fats, and carbohydrates forms the starting point, the A B C, as one might say, of chemical physiology.

I will conclude this introductory chapter by giving a list of the apparatus and reagents necessary for a practical study of the subject, and some tables which it will be often found convenient to refer to.

The following set of reagents conveniently contained in 4 to 6 oz. glass stoppered bottles should be provided for each two students:—

1. Sulphuric acid, concentrated.
2. " " 25 per cent.
3. " " 0·1 "
4. Nitric acid, concentrated.
5. Fuming nitric acid.
6. Hydrochloric acid, concentrated.
7. " " 0·2 per cent.<sup>1</sup>
8. Acetic acid, glacial.
9. " " 20 per cent.
10. " " 2 "
11. Caustic potash, 20 per cent.
12. " " 0·1 "
13. Ammonia.
14. Sodium carbonate, 1 per cent.
15. Ammonium sulphide solution.
16. Silver nitrate, 1 per cent.
17. Barium chloride, saturated solution.
18. Aminonium molybdate solution.
19. Millon's reagent.<sup>2</sup>
20. Solution of ferrocyanide of potassium.
21. " litmus.
22. " sodium phosphate.
23. " iodine in potassium iodide.
24. Methylated spirit.
25. Ether.
26. Esbach's reagent.<sup>3</sup>
27. Solution of copper sulphate, 1 per cent.

The following additional reagents will be required by those taking the advanced course:—

28. Solution of mercuric chloride.
29. " potassium ferricyanide.
30. Sodium carbonate, saturated solution.

<sup>1</sup> Made by adding 94 c.c. of water to 6 c.c. of the concentrated hydrochloric acid of the British Pharmacopœia.

<sup>2</sup> Mercury is dissolved in its own weight of strong nitric acid. The solution so obtained is diluted with twice its volume of water. The decanted clear liquid is Millon's reagent.

<sup>3</sup> Ten grammes of picric acid and 20 grammes of citric acid are dissolved in 800 to 900 c.c. of boiling water, and then sufficient water added to make up a litre.

31. Sodium chloride, saturated solution
32. " " 10 per cent. solution
33. Magnesium sulphate, saturated "
34. Ammonium " " "
35. Lime water.
36. Baryta mixture.<sup>1</sup>
37. Sodium acetate solution.<sup>2</sup>
38. Phosphoric acid, 0·5 per cent.
39. Absolute alcohol.

In addition to these, there should be kept in stock in the laboratory, to be given out for the lessons in which they are used, the following:—

1. Solid sodium chloride.
2. " magnesium sulphate.
3. " ammonium "
4. " sodo-magnesium sulphate.
5. Standard solution of uranium acetate or nitrate for estimating phosphates.<sup>3</sup>
6. Standard solution of mercuric nitrate for estimating urea.
7. " " " silver " " " chlorides.
8. " " " " " "
9. Fehling's solution.
10. Caustic soda, 40 per cent.
11. Bromine.
12. Solution of potassium bichromate.
13. Phenyl hydrazine hydrochloride.
14. Solid sodium acetate.
15. Phospho-tungstic acid.
16. Glacial phosphoric acid.

Each student should be provided with—

1. A bunsen burner.
2. 1 dozen test-tubes in test-tube stand.
3. 2 or 3 4-oz. flasks.
4. 2 flat porcelain dishes.
5. 2 or 4 4-oz. beakers.
6. 2 small glass funnels and a funnel stand.
7. A glass stirring-rod and a small pipette.
8. 1 burette.
9. An iron tripod with wire gauze.
10. Filter papers and litmus papers.
11. A 100-c.c. cylindrical measuring glass.
12. A thermometer marked in degrees Centigrade.
13. A urinometer.
14. A tin can on a stand to be used as a water-bath.

Apparatus which is not so frequently used, such as that employed in generating carbonic anhydride, carbonic oxide, or sulphuretted hydrogen, may be given out as required. The laboratory should also possess a good balance, with its accessories, water and air baths, kept at various temperatures, retorts, and analytical apparatus generally. The microscope, polarimeter, spectroscope, dialyser, are also frequently employed in chemico-physiological investigations.

<sup>1</sup> Made by mixing 1 volume of barium nitrate solution with 2 of barium hydrate solution, both saturated *in the cold*.

<sup>2</sup> Prepared as follows:—Sodium acetate, 100 grammes; water, 900 c.c.; glacial acetic acid, 100 c.c.

<sup>3</sup> Instructions how to make standard solutions will be given in the lessons where they are used.

## WEIGHTS AND MEASURES

The weights and measures usually employed in science are those of the metric system, but as in this country the practical physician still largely uses English grains and ounces, we may compare the two systems in the following way :—

### *Weights.*

#### (English System.)

1 grain	=	0·0648 gramme
1 ounce = 437·5 grains	=	28·3595 grammes
1 lb. = 16 oz. = 7,000 grains	=	453·5925 "

The scruple = 20 grains = 1·296 gramme, and the drachm = 60 grains = 3·888 grammes, are retained in use, but neither is an aliquot part of the ounce; though for practical purposes an ounce is considered to consist of 8 drachms.

#### (Metric System.)

1 milligramme	=	0·001 gramme	=	0·015432 grain
1 centigramme	=	0·01	=	0·154323 "
1 decigramme	=	0·1	=	1·543235 "
1 gramme	=		=	15·43235 grains
1 decagramme	= 10	grammes	=	154·3235 "
1 hectogramme	= 100	"	=	1548·235 "
1 kilogramme	= 10000	"	=	15432·35 "
				= 2 lb. 3 oz. 119·8 "

### *Measures of Length.*

#### (English System.)

1 inch	= 25·4	millimetres
1 foot	= 12 inches	= 304·8 millimetres

#### (Metric System.)

The standard of length is the metre; subdivisions and multiples of which, with the prefixes milli-, centi-, and deci- on the one hand, and deca-, hecto-, and kilo- on the other, have the same relation to the metre as the subdivisions and multiples of the gramme, in the table just given, have to the gramme, thus :

1 millimetre	= 0·001	metre	= 0·03937	inch
1 centimetre	= 0·01	"	= 0·3937	"
1 decimetre	= 0·1	"	= 3·93707	inches
1 metre			= 39·37079	"

### *Measures of Capacity.*

#### (English System.)

1 minim	=	0·059	cubic centimetre
1 fluid drachm = 60 minims	=	3·549	cubic centimetres
1 fluid ounce = 8 fluid drachms	=	28·398	"
1 pint = 20 fluid ounces	=	567·936	"
1 gallon = 8 pints	=	4·54837	litres

## (Metric System.)

In the metric system the measures of capacity are intimately connected with the measures of length; we thus have cubic millimetres, cubic centimetres, and so forth. The standard of capacity is the litre, which is equal to 1,000 cubic centimetres; and each cubic centimetre is the volume of 1 gramme of distilled water at 4° C.<sup>1</sup>

1 cubic centimetre (generally written c.c.) = 16.931 minims.  
 1 litre = 1,000 c.c. = 1 pint 15 oz. 2 drs. 11 m. = 35.2154 fluid ounces.  
 1 cubic inch = 16.386 c.c.

## THERMOMETRIC SCALES

The scale most frequently used in this country is the Fahrenheit scale; in this the freezing point of water is 32°, and the boiling point 212°. On the Continent the Réaumur scale is largely employed, in which the freezing point is 0°, and the boiling point 80°. In scientific work the Centigrade scale has almost completely taken the place of these; in this system the freezing point is 0°, and the boiling point 100°.

To convert degrees Fahrenheit into degrees Centigrade, subtract 32 and multiply by  $\frac{5}{9}$ , or  $C = (F - 32)\frac{5}{9}$ . Conversely, degrees Centigrade may be converted into degrees Fahrenheit by the following formula :  $F = \frac{9}{5}C + 32$ .

## TENSION OF AQUEOUS VAPOUR IN MILLIMETRES OF MERCURY FROM 10° TO 25° C.

10°. 9.126	14°. 11.882	18°. 15.351	22°. 19.675
11°. 9.751	15°. 12.677	19°. 16.345	23°. 20.909
12°. 10.421	16°. 13.519	20°. 17.396	24°. 22.211
13°. 11.130	17°. 14.409	21°. 18.505	25°. 23.582

## TABLE OF THE DENSITY OF WATER AT TEMPERATURES BETWEEN 0° AND 30° C.

0°. 0.99988	8°. 0.99988	16°. 0.99900	24°. 0.99738
1°. 0.99993	9°. 0.99982	17°. 0.99884	25°. 0.99714
2°. 0.99997	10°. 0.99974	18°. 0.99866	26°. 0.99689
3°. 0.99999	11°. 0.99965	19°. 0.99847	27°. 0.99662
4°. 1.00000	12°. 0.99955	20°. 0.99827	28°. 0.99635
5°. 0.99999	13°. 0.99942	21°. 0.99806	29°. 0.99607
6°. 0.99997	14°. 0.99930	22°. 0.99785	30°. 0.99579
7°. 0.99994	15°. 0.99915	23°. 0.99762	

<sup>1</sup> 4° C. is the temperature at which water has the greatest density. For practical purposes measures are more often constructed so that a cubic centimetre holds a gramme of water at 16° C., which is about the average temperature of rooms. The true cubic centimetre contains only 0.999 gramme at 16° C.

## SYMBOLS AND ATOMIC WEIGHTS OF THE PRINCIPAL ELEMENTS

Aluminium	Al	27.02	Fluorine	Fl	19.1	Phosphorus	P	30.96
Antimony	Sb	120.0	Gold	Au	197.0	Platinum	Pt	194.3
Arsenic	As	74.9	Hydrogen	H	1.0	Potassium	K	39.04
Barium	Ba	136.8	Iodine	I	126.53	Silver	Ag	107.66
Bismuth	Bi	208.0	Iron	Fe	55.9	Silicon	Si	28.3
Boron	Bo	10.9	Lead	Pb	206.4	Sodium	Na	23.0
Bromine	Br	79.75	Magnesium	Mg	24.0	Strontium	Sr	87.3
Cadmium	Cd	112.0	Manganese	Mn	55.0	Sulphur	S	31.98
Calcium	Ca	39.9	Mercury	Hg	199.8	Tin	Sn	{ 64.9
Carbon	C	11.97	Nickel	Ni	58.6			65.48
Chlorine	Cl	35.37	Nitrogen	N	14.01	Tungsten	W	183.6
Copper	Cu	63.2	Oxygen	O	15.96	Zinc	Zn	117.8

# ELEMENTARY COURSE

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## LESSON I

### *THE CARBOHYDRATES AND FATS*

1. Note the general appearance of the specimens of grape sugar or dextrose, dextrin, and starch which are given round.
2. Put some of each into cold water. Starch is insoluble; dextrose and dextrin dissolve after a time, but more readily in hot water.
3. *Trommer's test for dextrose*.—Put a few drops of copper sulphate solution into a test-tube, then solution of dextrose, and then strong caustic potash. On adding the caustic potash a precipitate is first formed, which, on adding more potash, redissolves, forming a blue solution. On boiling this a yellow or red precipitate (cuprous hydrate or oxide) forms.
4. *Dextrin*.—Add iodine solution to solution of dextrin, and a reddish-brown solution is produced. The colour disappears on heating.
5. *Starch*.—(a) Examine microscopically the scrapings from the surface of a freshly cut potato. Note the appearance of the starch grains with their concentric markings.  
 (b) On boiling starch with water an opalescent solution is formed, which, if strong, gelatinises on cooling.  
 (c) Add iodine solution. An intense blue colour is produced, which disappears on heating, and if not heated too long reappears on cooling.  
 N.B.—Prolonged heating drives off the iodine, and consequently no blue colour returns after cooling.  
 (d) Conversion into dextrin and dextrose. To some starch solution in a flask add a few drops of 25-per-cent. sulphuric acid, and boil for 15 minutes. Take some of the liquid, which is now clear, and test for dextrin and dextrose.
6. *Glycogen*.—Solution of glycogen is given round: (a) it is opalescent like that of starch.  
 (b) With iodine it gives a brown colour like dextrin.  
 (c) By boiling with 25-per-cent. sulphuric acid it is converted into grape sugar.
7. Lard is given round as an example of a fat. It is insoluble in water. By boiling with potash it yields a solution of soap.
8. Add to this solution a few drops of 25-per-cent. sulphuric acid. On heating a layer of fatty acid collects on the surface.

## CARBOHYDRATES

The carbohydrates are found chiefly in vegetable tissues, and many of them form important foods. Some carbohydrates are, how-

ever, found in or formed by the animal organism. The most important of these are *glycogen*, or animal starch; *dextrose*; and *lactose*, or milk sugar.

The carbohydrates may be conveniently defined as compounds of carbon, hydrogen, and oxygen, the two last named elements being in the proportion in which they occur in water.

They may be for the greater part arranged into three groups according to their empirical formulæ. The names and formulæ of these groups, and the most important members of each, are as follows:—

1. Glucoses, $C_6H_{12}O_6$	2. Sucroses, or Saccharoses, $C_{12}H_{22}O_{11}$	3. Amyloses, $(C_6H_{10}O_6)_n$
+ Dextrose - Levulose + Galactose	+ Cane sugar + Lactose + Maltose	+ Starch + Glycogen + Dextrin Cellulose Gums

The + and - signs in the above list indicate that the substances to which they are prefixed are dextro- and levo-rotatory respectively as regards polarised light (see Appendix).

The formulæ given above are merely empirical; and there is no doubt that the quantity  $n$  in the starch group is variable and often large; hence the name polysaccharides that Tollens gives to the group. Research has, moreover, shown that the glucoses are either aldehydes (dextrose) or ketones (levulose) of the hexatomic alcohol mannite. The amyloses may be regarded as the anhydrides of the glucoses  $[nC_6H_{12}O_6 - nH_2O = (C_6H_{10}O_5)_n]$ . The sucroses are condensed glucoses—i.e. they are formed by the combination of two molecules of glucose with the loss of one molecule of water ( $C_6H_{12}O_6 + C_6H_{12}O_6 - H_2O = C_{12}H_{22}O_{11}$ ). The following are the chief facts in relation to each of the principal carbohydrates.

**Dextrose or Grape Sugar.**—This carbohydrate is found in fruits, honey, and in minute quantities in the blood and numerous tissues, organs, and fluids of the body. It is the form of sugar found in large quantities in the blood and urine in the disease known as diabetes.



FIG. 1.—Dextrose crystals.

Dextrose is soluble in hot and cold water and in alcohol. It is crystalline (see fig. 1), but not so sweet as cane sugar. When heated with strong potash certain complex acids are formed which have

a yellow or brown colour. This constitutes *Moore's test* for sugar. In alkaline solutions dextrose reduces salts of silver, bismuth, mercury, and copper. The reduction of cupric to cuprous salts constitutes *Trommer's test*, which has been already described at the head of the lesson. Another important property of grape sugar is that under the influence of yeast it is converted into alcohol and carbonic acid ( $C_6H_{12}O_6 = 2C_2H_6O + 2CO_2$ ).

Dextrose may be estimated by the fermentation test, by the polarimeter, and by the use of Fehling's solution. The last method is the most important : it rests on the same principles as Trommer's test, and we shall study it in connection with diabetic urine (see Lesson XII.)

**Levulose.**—When cane sugar is treated with dilute mineral acids it undergoes a process known as inversion—*i.e.* it takes up water and is converted ~~with~~ equal parts of dextrose and levulose. The previously dextro-rotatory solution of cane sugar then becomes levo-rotatory, the levo-rotatory power of the levulose being greater than the dextro-rotatory power of the dextrose formed. Hence the term *inversion*. Similar hydrolytic changes are produced by certain ferments, such as the invert ferment of the intestinal juice.

Pure levulose can be crystallised, but so great is the difficulty of obtaining crystals of it that one of its names was uncrystallisable sugar. Small quantities of levulose have been found in blood, urine, and muscle. It has been recommended as an article of diet in diabetes in place of ordinary sugar ; in this disease it does not appear to have the harmful effect that other sugars produce. Levulose gives the same general reactions as dextrose.

**Galactose** is formed by the action of dilute mineral acids or inverting ferments on lactose or milk sugar. It resembles dextrose in being dextro-rotatory, in reducing cupric salts in Trommer's test, and in being directly fermentable with yeast. When oxidised by means of nitric acid it, however, yields an acid called mucic acid ( $C_6H_{10}O_8$ ), which is only sparingly soluble in water. Dextrose when treated this way yields an isomeric acid—*i.e.* an acid with the same empirical formula, called saccharic acid, which is readily soluble in water.

**Inosite**, or muscle sugar, is found in muscle, kidney, liver, and other parts of the body in small quantities. It is also largely found in the vegetable kingdom. It is a crystallisable substance (see fig. 2) and has the same formula ( $C_6H_{12}O_6$ ) as the glucoses. It is, however, not a sugar. It gives none of the sugar tests, and careful analysis has shown it has quite a different chemical constitution from the true sugars.

**Cane Sugar.**—This sugar is generally distributed throughout the

vegetable kingdom in the juices of plants and fruits, especially the sugar cane, beetroot, mallow, and sugar maple. It is a substance of

great importance as a food. After abundant ingestion of cane sugar traces may appear in the urine, but the greater part undergoes inversion in the alimentary canal.

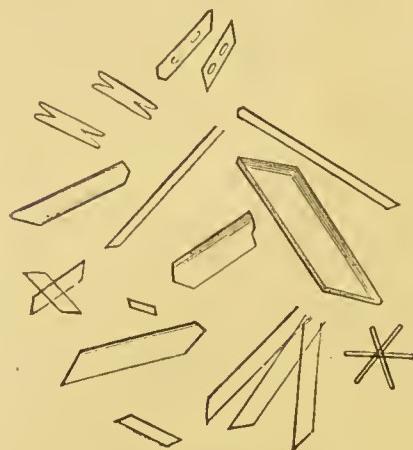
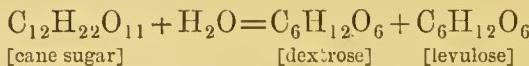


FIG. 2.—Inosite crystals.

acids, or by means of inverting ferment, such as that occurring in the succus entericus, it undergoes inversion—that is, it takes up water and is split into equal parts of dextrose and levulose (see p. 11).



With yeast, cane sugar is first inverted by means of a special soluble ferment produced by the yeast cells, and then there is an alcoholic fermentation of the glucoses so formed.

**Lactose**, or **milk sugar**, occurs in milk. It has also been described as occurring in the urine of women in the early days of lactation or after weaning.

It crystallises in rhombic prisms (see fig. 3). It is much less soluble in water than cane sugar or dextrose, and has only a slightly sweet taste. It is insoluble in alcohol and ether; aqueous solutions are dextro-rotatory.

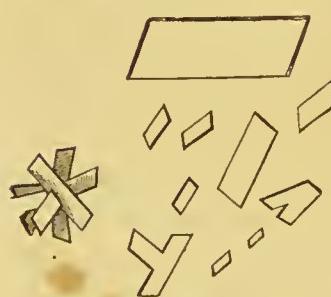
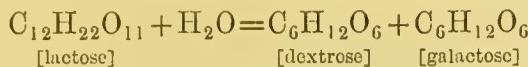


FIG. 3.—Milk-sugar crystals.

quantity of Fehling's solution, it would require ten parts of a solution of lactose of the same strength to reduce the same quantity of Fehling's solution.

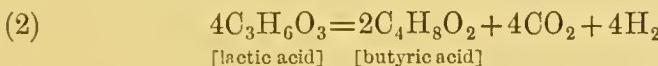
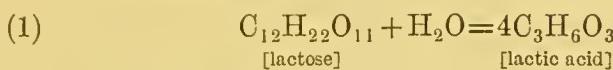
Solutions of lactose give Trommer's test, but when the reducing power is tested quantitatively by Fehling's solution it is found to be a less powerful reducing agent than dextrose. If it required seven parts of a solution of dextrose to reduce a given

Lactose, like cane sugar, can be hydrolysed by the same agencies as those already enumerated in connection with cane sugar. The glucoses formed are dextrose and galactose.



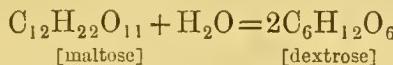
With yeast it is first inverted, and then alcohol is formed. This, however, occurs slowly.

With the lactic-acid organisms which bring about the souring of milk the lactic-acid fermentation is produced. This may also occur as the result of the action of putrefactive bacteria in the alimentary canal. The two stages of the lactic-acid fermentation are represented by the following equations :—



**Maltose** is the end product of the action of malt diastase on starch, and is also formed as an intermediate product in the action of dilute sulphuric acid on the same substance. It is also the chief sugar formed from starch by the diastatic ferments contained in the saliva (ptyalin) and pancreatic juice (amylopsin). It can be obtained in the form of acicular crystals; it is strongly dextro-rotatory. It gives Trommer's test; but its reducing power, as measured by Fehling's solution, is one-third less than that of dextrose.

By prolonged boiling with water, or, more readily, by boiling with a dilute mineral acid, or by means of an inverting ferment, such as occurs in the intestinal juice, it is converted into dextrose.



It undergoes readily the alcoholic fermentation.

**Starch** is widely diffused through the vegetable kingdom. It occurs in nature in the form of microscopic grains, varying in size and appearance, according to their source. Each consists of a central spot, round which more or less concentric envelopes of starch proper or granulose alternate with layers of cellulose. Cellulose has very little digestive value, but starch is a most important food.

Starch is insoluble in cold water: it forms an opalescent solution in boiling water, which if concentrated gelatinises on cooling. Its most characteristic reaction is the blue colour it gives with iodine.

On heating starch with mineral acids, dextrose is formed. By the

action of diastatic ferments, maltose is the chief end product. In both cases dextrin is an intermediate stage in the process.

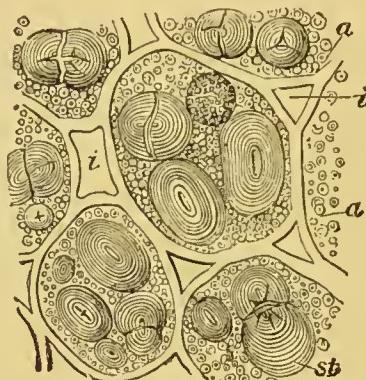


FIG. 4.—Section of pea showing starch and aleurone grains embedded in the protoplasm of the cells. (Yeo, after Sachs) : *a*, aleurone grains; *st*, starch grains; *i*, intercellular spaces.

Before the formation of dextrin the starch solution loses its opalescence, a substance called *soluble starch* being formed. This, like native starch, gives a blue colour with iodine. Although the molecular weight of starch is unknown, the formula for soluble starch is probably  $5(C_{12}H_{20}O_{10})_2$ . Equations that represent the formation of sugars and dextrans from this are very complex, and are at present only theoretical.

**Dextrin** is the name given to the intermediate products in the hydration of starch, and two chief varieties are distinguished:—*erythro-dextrin*, which gives a reddish-brown colour with iodine; and *achroo-dextrin*, which does not.

It is readily soluble in water, but insoluble in alcohol and ether. It is gummy and amorphous. It does not give Trommer's test, nor does it ferment with yeast. It is dextro-rotatory. By hydrating agencies it is converted into glucose.

**Glycogen**, or animal starch, is found in liver and muscle. It is also abundant in all embryonic tissues.

Glycogen is a white tasteless powder, soluble in hot water, forming, like starch, an opalescent solution. It is insoluble in alcohol and ether. It is dextro-rotatory. With Trommer's test it gives a blue solution, but no reduction occurs on boiling.

With iodine it gives a reddish or port-wine colour, very similar to that given by dextrin. Dextrin may be distinguished from glycogen by (1) the fact that it gives a clear, not an opalescent, solution with water; and (2) it is not precipitated by basic lead acetate as glycogen is. It is, however, precipitated by basic lead acetate and ammonia.

(3) Glycogen is precipitated by 60 per cent. of alcohol ; the dextrins require 85 per cent. or more.

**Cellulose.**—This is the colourless material of which the cell-walls and woody fibres of plants are composed. By treatment with strong mineral acids it is, like starch, converted into glucose, but with much greater difficulty. The various digestive ferments have little or no action on cellulose ; hence the necessity of boiling starch before it is taken as food. Boiling bursts the cellulose envelopes of the starch grains, and so allows the digestive juice to get at the starch proper.

Cellulose is found in a few animals, as in the test or outer investment of the Tunicates.

### THE FATS

Fat is found in small quantities in many animal tissues. It is, however, found in large quantities in two situations, viz. adipose

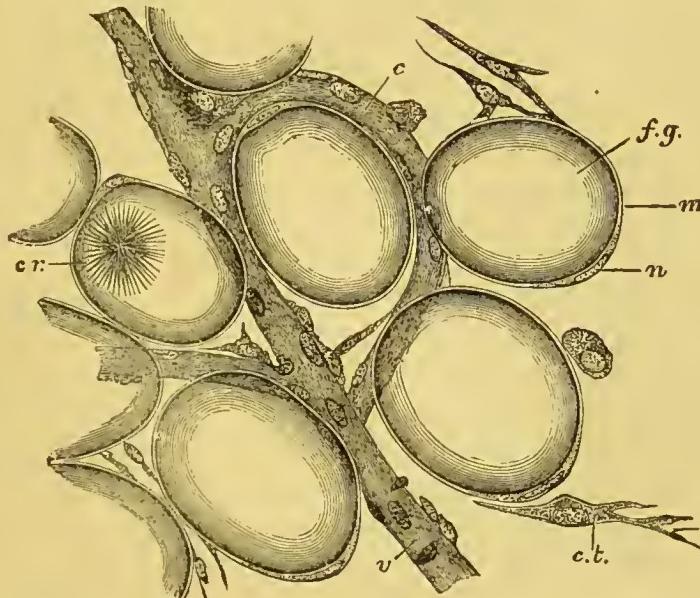


FIG. 5.—A few cells from the margin of a fat lobule : *f.g.*, fat globule distending fat cell ; *n*, nucleus ; *m*, membranous envelope of the cell ; *c r.*, bunch of crystals within a fat cell ; *c*, capillary vessel ; *v*, venule ; *c t.*, connective tissue cell. The fibres of the connective tissue are not represented.

tissue and milk. The consideration of the fat in milk is postponed to Lesson IV.

The contents of the fat cells of adipose tissue are fluid during life, the normal temperature of the body ( $36^{\circ}$  C., or  $99^{\circ}$  F.) being considerably above the melting-point ( $25^{\circ}$  C.) of the mixture of the fats found there. These fats are three in number, and are called palmitin, stearin, and olein. They differ from one another in chemical composition and in certain physical characters, such as melting-point and

solubilities. Olein melts at 0° C., palmitin at 45° C., and stearin at 53–66° C. It is thus olein which holds the other two dissolved at the body temperature. Fats are all soluble in hot alcohol, ether, and chloroform, but insoluble in water.

**Chemical Constitution of the Fats.**—The fats are compounds of fatty acids with glycerin, and may be termed glycerides. The term *hydrocarbon*, applied to them by some authors, is wholly incorrect.

The fatty acids form a series of acids derived from the monatomic alcohols by oxidation. Thus, to take ordinary ethyl alcohol,  $C_2H_6O$ , the first stage in oxidation is the removal of two atoms of hydrogen to form aldehyde,  $C_2H_4O$ ; on further oxidation an atom of oxygen is added to form acetic acid,  $C_2H_4O_2$ .

A similar acid can be obtained from all the other alcohols, thus :—

From methyl alcohol  $CH_3.HO$ , formic acid  $H.COOH$  is obtained.

„ ethyl „	$C_2H_5.HO$ ,	acetic „	$CH_3.COOH$	„
„ propyl „	$C_3H_7.HO$ ,	propionic „	$C_2H_5.COOH$	„
„ butyl „	$C_4H_9.HO$ ,	butyric „	$C_3H_7.COOH$	„
„ amyl „	$C_5H_{11}.HO$ ,	valeric „	$C_4H_9.COOH$	„

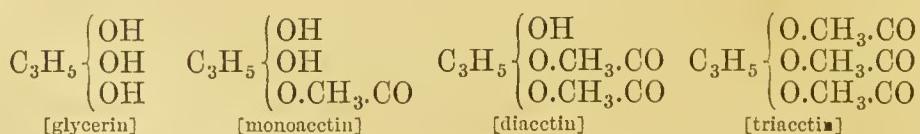
and so on.

Or in general terms :—

From the alcohol with formula  $C_nH_{2n+1}.HO$  the acid with formula  $C_{n-1}H_{2n-1}.CO.OH$  is obtained. The sixteenth term of this series has the formula  $C_{15}H_{31}.CO.OH$ , and is called **palmitic acid**; the eighteenth has the formula  $C_{17}H_{35}.CO.OH$ , and is called **stearic acid**. Each acid, as will be seen, consists of a radicle,  $C_{n-1}H_{2n-1}CO$ , united to hydroxyl ( $HO$ ).

**Oleic acid**, however, is not a member of the fatty acid series proper, but belongs to a somewhat similar series of acids known as the acrylic series, of which the general formula is  $C_{n-1}H_{2n-3}COOH$ . It is the eighteenth term of the series, and its formula is  $C_{17}H_{33}.CO.OH$ .

**Glycerin or Glycerol** is a triatomic alcohol,  $C_3H_5(HO)_3$ —i.e. three atoms of hydroxyl united to a radicle glyceryl ( $C_3H_5$ ). The hydrogen in the hydroxyl atoms is replaceable by other organic radicles. As an example take the radicle of acetic acid called acetyl ( $CH_3.CO$ ). The following formulæ represent the derivatives that can be obtained by replacing one, two, or all three hydroxyl hydrogen atoms in this way :—



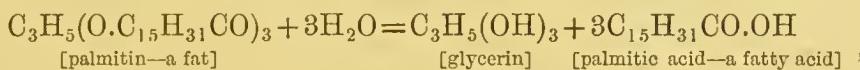
Triacetin is a type of a neutral fat; stearin, palmitin, and olein

ought more properly to be called tristearin, tripalmitin, and triolein respectively. Each consists of glycerin in which the three atoms of hydrogen in the hydroxyls are replaced by radicles of the fatty acid. This is represented in the following formulæ:—

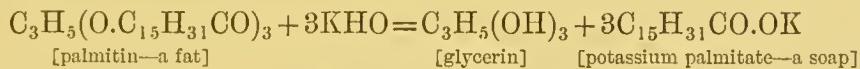
Acid	Radicle	Fat
Palmitic acid $C_{15}H_{31}\cdot COOH$	Palmityl $C_{15}H_{31}\cdot CO$	Palmitin $C_3H_5(O\cdot C_{15}H_{31}\cdot CO)_3$
Stearic acid $C_{17}H_{35}\cdot COOH$	Stearyl $C_{17}H_{35}\cdot CO$	Stearin $C_3H_5(O\cdot C_{17}H_{35}\cdot CO)_3$
Oleic acid $C_{17}H_{33}\cdot COOH$	Oleyl $C_{17}H_{33}\cdot CO$	Olein $C_3H_5(O\cdot C_{17}H_{33}\cdot CO)_3$

**Decomposition Products of the Fats.**—The fats split up into the substances out of which they are built up.

Under the influence of superheated steam, mineral acids, and in the body by means of certain ferments (for instance, the fat-splitting ferment steapsin of the pancreatic juice), a fat combines with water and splits into glycerin and the fatty acid. The following equation represents what occurs in a fat, taking tripalmitin as an example:—



In the process of saponification much the same sort of reaction occurs, the final products being glycerin and the palmitate of the base employed, which is called a soap. Suppose, for instance, that potassium hydrate is used; we get—



**Emulsification.**—Another change that fats undergo in the body is very different from saponification. It is a physical rather than a chemical change; the fat is broken up into very small globules, such as is seen in the natural *emulsion*—milk.

## LESSON II

## THE PROTEIDS

**1. Tests for Proteids.**—The following tests are to be tried with a mixture of one part of white of egg to ten of water. (Egg-white contains a mixture of albumin and globulin.)

(a) *Heat Coagulation*.—Faintly acidulate with a few drops of 2-per-cent. acetic acid and boil. The proteid is rendered insoluble (coagulated proteid).

(b) *Precipitation with Nitric Acid*.—The addition of strong nitric acid to the original solution also produces a white precipitate.

(c) *Xanthoproteic Reaction*.—On boiling the white precipitate produced by nitric acid it turns yellow; after cooling add ammonia; the yellow becomes orange.

(d) *Millon's Test*.—Millon's reagent (which is a mixture of the nitrates of mercury containing excess of nitric acid; see p. 4) gives a white precipitate, which turns brick-red on boiling.

(e) After the addition of acetic acid, potassium ferrocyanide gives a white precipitate.

(f) Add a drop of a 1-per-cent. solution of cupric sulphate to the original solution and then caustic potash, and a violet solution is obtained.

2. Repeat experiment (f) with a solution of commercial peptone, and note that a rose-red (biuret) reaction is obtained.

3. *Action of Neutral Salts*.—The following experiments may be performed with serum, which, like egg-white, contains albumin and globulin.

(a) Saturate it with magnesium sulphate by adding crystals of the salt to the serum and shaking vigorously in a flask. A white precipitate of serum-globulin is produced. Filter. The filtrate contains serum-albumin. (Serum-globulin is incompletely precipitated by diluting the serum with a large quantity of water and also by adding weak acetic acid to, or passing a stream of carbonic acid gas through diluted serum. See Lesson XXI.)

(b) Saturate similarly another portion with ammonium sulphate; a precipitate is produced of both the globulin and albumin. Filter. The filtrate contains no proteid.

4. Repeat the last experiment with a solution of commercial peptone. A precipitate is produced of the albumoses or proteoses it contains. Filter. The filtrate contains the true peptone. Ammonium sulphate precipitates all proteids except peptone.

---

The proteids are the most important substances that occur in animal and vegetable organisms; none of the phenomena of life occur without their presence; and though it is impossible to state positively that they occur as such in living protoplasm, they are invariably obtained by subjecting living structures to analytical processes.

Proteids are highly complex and (for the most part) uncrystallisable compounds of carbon, hydrogen, oxygen, nitrogen, and sulphur occurring in a solid viscous condition or in solution in nearly all the liquids and solids of the body. The different members of the groups present differences in chemical and physical properties. They all possess, however, certain common chemical reactions, and are united by a close genetic relationship.

The various proteids differ a good deal in elementary composition. Hoppe-Seyler gives the following percentages :—

	C	H	N	S	O
From	51·5	6·9	15·2	0·3	20·9
To	54·5	7·3	17·0	2·0	23·5

We are, however, not acquainted with the constitutional formula of proteid substances. There have been many theories on the subject, but practically all that is known with certainty is that many different substances may be obtained by the decomposition of proteids. How they are built up into the protcid molecule is unknown. The decompositions that occur in the body are, moreover, different from those which can be made to occur in the laboratory ; hence the conclusion that living protoplasm differs somewhat from the non-living proteid material obtainable from it.

(1) In the body. Carbonic acid, water, and urea are the chief final products. Glycocine, leucine, creatine, uric acid, &c. are probably intermediate products. Carbohydrates (glycogen) and fats may also originate from proteids.

(2) Outside the body. Various strong reagents break up proteids into ammonia, carbonic acid, amines, fatty acids, amido-acids like leucine and glycocine, and aromatic compounds like tyrosine.

### TESTS FOR PROTEIDS

**Solubilities.**—All proteids are insoluble in alcohol and ether. Some are soluble in water, others insoluble. Many of the latter are soluble in weak saline solutions. Some are insoluble, others soluble in concentrated saline solutions. It is on these varying solubilities that proteids are classified.

All proteids are soluble with the aid of heat in concentrated mineral acids and alkalis. Such treatment, however, decomposes as well as dissolves the proteid. Proteids are also soluble in gastric and pancreatic juices ; but here, again, they undergo a change, being converted into a hydrated variety of proteid called *peptone*. The intermediate substances formed in this process are called *proteoses* or

*albumoses.* Commercial peptone contains a mixture of proteoses and true peptone.

**Heat Coagulation.**—Many of the proteids which are soluble in water or saline solutions are rendered insoluble when those solutions are heated. This is true for most of the proteids that occur in nature. The solidifying of white of egg when heated is a familiar instance of this. The temperature of heat coagulation differs in different proteids : thus myosinogen and fibrinogen coagulate at about 56° C. ; serum albumin and serum globulin at about 75° C.

The proteids which are coagulated by heating their solutions come under two classes—the *albumins* and the *globulins*. The full distinction between these we shall see immediately. We may, however, state here that the albumins are soluble in distilled water ; the globulins are not, but require salts to hold them in solution.

**Indiffusibility.**—The proteids (peptones excepted) belong to the class of substances called *colloids* by Thomas Graham ; that is, they

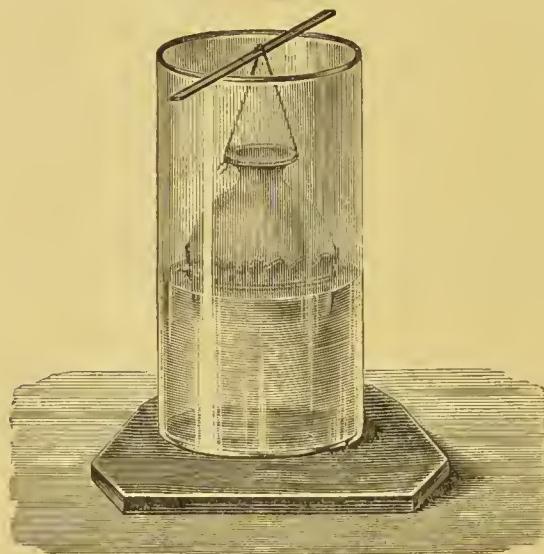


FIG. 6.—Dialyser. The lower opening of the bell jar suspended in water is tightly covered with parchment paper. The fluid to be dialysed is placed within this vessel ; the crystalloids pass out into the distilled water outside through the parchment paper.

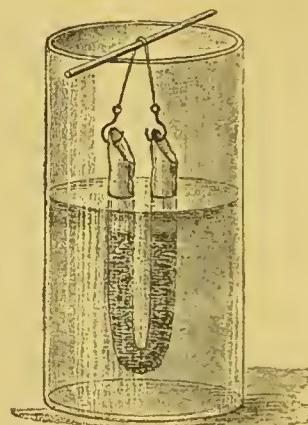


FIG. 7.—In this form of dialyser the substance to be dialysed is placed within the piece of tubing suspended in the larger vessel of water. The tubing is made of parchment paper.

pass with difficulty, or not at all through animal membranes. In the construction of dialysers, vegetable parchment is very largely used (see figs. 6 and 7).

Proteids may thus be separated from diffusible (*crystalloid*) substances like salts, but the process is a somewhat tedious one. If some serum be placed in a dialyser, and distilled water outside, the greater amount of the salts pass into the water through the mem-

brane ; the two proteids, serum albumin and serum globulin, remain inside. The serum globulin is, however, precipitated, as the salts which previously kept it in solution have been removed.

**Action on Polarised Light.**—All the proteids are levo-rotatory, but the amount of rotation they produce varies with the kind of proteid.

**Colour Reactions.**—The principal colour reactions : (1) the xanthoproteic ; (2) Millon's ; (3) the violet colour with copper sulphate and caustic potash have been already given in the heading to this lesson. Peptones behave differently from the native proteids in this last test. They give a rose-red colour instead of a violet, if only a trace of copper sulphate is used. The albumoses act in this respect like the peptones. This rose-red colour is also given by the substance called biuret ;<sup>1</sup> hence the test is often called the *biuret reaction*.

**Precipitants of Proteids.**—Proteids are precipitated by a large number of reagents ; the peptones and albumoses are exceptions in many cases, and will be considered separately afterwards (see Lesson VI.)

Solutions of the proteids are precipitated by—

1. Strong acids, like nitric acid.
2. Picric acid.
3. Acetic acid and potassium ferrocyanide.
4. Acetic acid and excess of neutral salts like sodium sulphate.
5. Salts of the heavy metals, like copper sulphate, mercuric chloride, lead acetate, silver nitrate, &c.
6. Tannin.
7. Alcohol.
8. Saturation with certain neutral salts, such as ammonium sulphate.

It is necessary that the words *coagulation* and *precipitation* should, in connection with the proteids, be carefully distinguished. The term *coagulation* is used when an insoluble proteid (coagulated proteid) is formed from a soluble one. This may occur—

1. When the proteid is heated—*heat coagulation*.
2. Under the influence of a ferment: for instance, when a curd is formed in milk by rennet or a clot in shed blood by the fibrin ferment—*ferment coagulation*.
3. When an insoluble precipitate is produced by the addition of certain reagents (nitric acid, picric acid, tannin, &c.)

There are, however, other precipitants of proteids in which the precipitate formed is readily soluble in suitable reagents, like saline solution, and the proteid continues to show its typical reactions. Such

<sup>1</sup> Biuret is formed by heating urea; ammonia passes off and leaves biuret, thus :—



precipitation is not coagulation. Such a precipitate is produced by saturation with ammonium sulphate. Certain proteids, called globulins, are more readily precipitated by such means than others. Thus, serum globulin is precipitated by half-saturation with ammonium sulphate. Full saturation with ammonium sulphate precipitates all proteids but peptone. The globulins are precipitated by certain salts, like sodium chloride and magnesium sulphate, which do not precipitate the albumins.

The precipitation produced by alcohol is peculiar in that after a time it becomes a coagulation. Proteid freshly precipitated by alcohol is readily soluble in water or saline media ; but after it has been allowed to stand some weeks under alcohol it becomes more and more insoluble. Albumins and globulins are most readily rendered insoluble by this method ; albumoses and peptones are apparently never rendered insoluble by the action of alcohol. This fact is of value in the separation of these proteids from others.

### CLASSIFICATION OF PROTEIDS

Both animal and vegetable proteids can be divided into the following six classes. We shall, however, be chiefly concerned with the animal proteids.

**Class I. Albumins.**—These are soluble in water, in dilute saline solutions, and in saturated solutions of sodium chloride and magnesium sulphate. They are, however, precipitated by saturating their solutions with ammonium sulphate. Their solutions are coagulated by heat, usually at 70–73° C. The following are instances :—

- (a) Serum albumin. Not precipitated by ether.
- (b) Egg albumin. Precipitated by ether.
- (c) Lact-albumin (see MILK).

**Class II. Globulins.**—These are insoluble in water, soluble in dilute saline solutions, and insoluble in concentrated solutions of neutral salts like sodium chloride, magnesium sulphate, and ammonium sulphate. A globulin dissolved in a dilute saline solution may therefore be precipitated—

1. By removing the salt—by dialysis (see p. 20).
2. By increasing the amount of salt. Magnesium sulphate added to saturation is the best to employ.

The globulins are coagulated by heat ; the temperature of heat coagulation varies considerably. The following are instances :—

- (a) Fibrinogen
- (b) Serum globulin (paraglobulin) } in blood plasma.

- (c) Globin, the proteid constituent of haemoglobin.
- (d) Myosinogen in muscle.
- (e) Crystallin in the crystalline lens.

If we compare together these two classes of proteids, the most important of the native proteids, we find that they all give the same general tests, that all are coagulated by heat, but that they differ in solubilities. This difference in solubility may be stated in tabular form as follows :—

	Water	Dilute saline solution	Saturated saline (NaCl, or MgSO <sub>4</sub> ) solution
Albumins . . .	soluble	soluble	soluble
Globulins . . .	insoluble	soluble	insoluble

The remaining classes of proteids we shall consider more fully in subsequent lessons.

### Class III. Albuminates.

- (a) Acid albumin or syntoinin.
- (b) Alkali albumin.  
(See next lesson.)

Class IV. Proteoses } These products of digestion will be studied  
Class V. Peptones } in Lessons VI. and VII.

Class VI. Coagulated Proteids.—There are two subdivisions of these :—

(a) Proteids in which coagulation has been produced by heat ; they are insoluble in water, saline solutions, weak acids, and weak alkalis ; soluble after prolonged boiling in concentrated mineral acids ; dissolved by gastric and pancreatic juices, they give rise to peptones.

- (b) Proteids in which coagulation has been produced by ferments :—
  - i. Fibrin (see BLOOD).
  - ii. Myosin (see MUSCLE).
  - iii. Casein (see MILK).
  - iv. Gluten (see FLOUR).

Tables illustrating the methods of testing for and separating proteids will be found at the end of this elementary course.

## LESSON III

## THE ALBUMINATES AND ALBUMINOIDS

1. *Action of Acids and Alkalies on Albumin.*—Take three test-tubes and label them A, B, and C.

In each place an equal amount of diluted egg-white, similar to that used in the last lesson.

To A add a few drops of 0·1-per-cent. solution of caustic potash.

To B add the same amount of 0·1-per-cent. solution of caustic potash.

To C add a rather larger amount of 0·1-per-cent. sulphuric acid.

Put all three into the warm bath<sup>1</sup> at about the temperature of the body (36–40° C.)

After five minutes remove test-tube A, and boil. The proteid is no longer coagulated by heat, having been converted into *alkali-albumin*. After

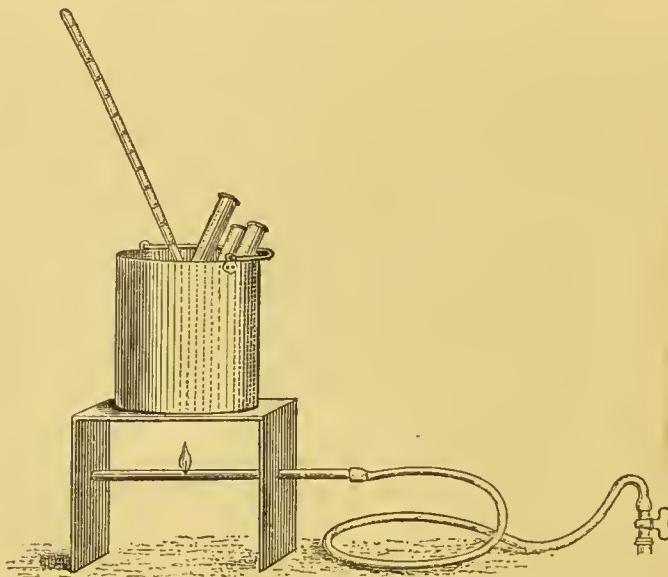


FIG. 8.—Simple warm bath, as described in footnote.

cooling, colour with litmus solution and neutralise with 0·1-per-cent. acid. At the neutral point a precipitate is formed which is soluble in excess of either acid or alkali.

Next remove B. This also now contains alkali-albumin. Add to it a few

<sup>1</sup> A convenient form of warm bath suitable for class purposes may be made by placing an ordinary tin pot half full of water over a bent piece of iron which acts as a warm stage as in the figure. The stage is kept warm by a small gas flame. Such a warm bath may be placed between every two or three students.

drops of sodium phosphate, colour with litmus, and neutralise as before. Note that the alkali-albumin now requires more acid for its precipitation than in A, the acid which is first added converting the sodium phosphate into acid sodium phosphate.

Now remove C from the bath. Boil it. Again there is no coagulation, the proteid having been converted into *acid-albumin*, or *syntonin*. After cooling, colour with litmus and neutralise with 0·1-per-cent. alkali. At the neutral point a precipitate is formed soluble in excess of acid or alkali. (Acid-albumin is formed more slowly than alkali-albumin, so it is best to leave this experiment to the last.)

2. Take some gelatin and dissolve it in hot water. On cooling, the solution sets into a jelly (gelatinisation).

3. Add a few drops of acetic acid to some saliva. A stringy precipitate of mucus is formed.

4. A tendon has been soaked for a few days in lime water. The fibres are not dissolved, but they are loosened from one another owing to the solution of the interstitial or ground substance by the lime water. Take some of the lime-water extract and add acetic acid. A precipitate of mucus is obtained. The fibres themselves consist of collagen, which yields gelatin on boiling.

## ALBUMINATES

**Albuminates** are compounds of proteid with mineral substances. Thus, if a solution of copper sulphate is added to a solution of albumin, a precipitate of copper albuminate is obtained. Similarly, by the addition of other salts of the heavy metals, other metallic albuminates are obtainable.

The albuminates which are obtained by the action of dilute acids and alkalis on either albumins or globulins are, however, of greater physiological interest, and it is to these we shall chiefly confine our attention. The general properties of the *acid-albumin*, or *syntonin*, and the *alkali-albumin* which are thereby respectively formed will be gathered from the practical exercise which stands at the head of this lesson. They are insoluble in pure water, but are soluble in either acid or alkali, and are precipitated by neutralisation unless disturbing influences like the presence of sodium phosphate are present. It may also be added that, like globulins, they are precipitated by saturation with such neutral salts as sodium chloride and magnesium sulphate. They are not coagulated by heat.

A variety of alkali albumin (probably a compound containing a large quantity of alkali) may be formed by adding strong potash to undiluted white of egg. The resulting jelly is called Lieberkühn's jelly.

Caseinogen, the chief proteid in milk, was at one time regarded as being a native alkali-albumin. It certainly is precipitated by acid, but we shall find that there are several reasons why it is no longer considered an alkali-albumin.

## ALBUMINOIDS

The albuminoids are a group of substances which, though similar to the proteids in many particulars, differ from them in certain other points. The principal members of the group are the following :—

1. **Collagen**, the substance of which the white fibres of connective tissue are composed. Some observers regard it as the anhydride of gelatin.

2. **Ossein**. This is the same substance, derived from bone.

3. **Gelatin**. This substance is produced by boiling collagen with water. It possesses the peculiar property of setting into a jelly when a solution made with hot water cools. It gives most of the proteid colour tests. Many observers state, however, that it contains no sulphur. On digestion it is like proteid converted into peptone-like substances, and is readily absorbed. Though it will replace in diet a certain quantity of proteid, acting as what is called a 'proteid-sparing' food, it cannot altogether take the place of proteid as a food. Animals fed on gelatin instead of proteid waste rapidly. *Chondrin*, the very similar substance obtained from hyaline cartilage, appears to be a mixture of gelatin with mucinoid materials.

4. **Mucin**. This is a widely-distributed substance, occurring in epithelial cells, or shed out by them (mucus, mucous glands, goblet cells); and in connective tissue where it forms the chief constituent of the ground substance or intercellular material.

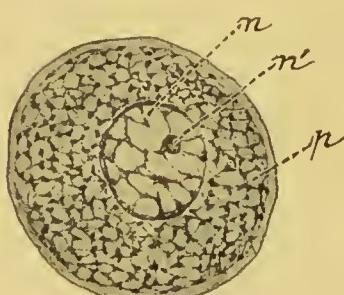
The mucin obtained from different sources varies in composition and reactions. There are probably several mucins: they all agree in the following points :—

(a) Physical character. Viscid and tenacious.

(b) Precipitability from solutions by acetic acid: they all dissolve in dilute alkalis like lime water.

(c) They are all compounds of a protein with a carbohydrate called animal gum, which by treatment with dilute mineral acid can be hydrated into a reducing but non-fermentable sugar.

FIG. 9.—Diagram of a cell: *p*, protoplasm composed of spongioplasm and hyaloplasm; *n*, nucleus with intranuclear network of chromatin or nuclein; and *n'*, nucleolus. (Schäfer.)



5. **Elastin**. This is the substance of which the yellow or elastic fibres of connective tissue are composed. It is a very insoluble material. The sarcolemma of muscular fibres and certain basement membranes are very similar.

6. **Nuclein**, the chief constituent of cell-nuclei. A similar substance

is also found in milk and yolk of egg. Its physical characters are something like mucin, but it differs chemically in containing a high percentage of phosphorus. Nuclein appears to be identical with the chromatin of histologists (see fig. 9).

**7. Nucleo-albumin.**—Compounds of proteids with nuclein. They are found in the protoplasm of cells. Caseinogen of milk and vitellin of egg-yolk are similar substances. In physical characters they often closely simulate mucin; in fact, the nucleo-albumin of bile is still frequently called mucin. They may be distinguished from mucin by the fact that they do not yield a reducing sugar on treatment with mineral acid; they yield on gastric digestion not only peptone but also an insoluble residue of nuclein which is soluble in alkalis, precipitable by acetic acid from such a solution, and contains a high percentage of phosphorus.

Some of the nucleo-albumins also contain iron, and it is probable that the normal supply of iron to the body is contained in the nucleo-albumins, or haemoglobins (Bunge), of plant and animal cells.

**8. Keratin,** or horny material, is the substance found in the surface layers of the epidermis, in hairs, nails, hoofs, and horns. It is very insoluble, and chiefly differs from proteids in its high percentage of sulphur. A similar substance, called *neurokeratin*, is found in neuroglia and nerve fibres. In this connection it is interesting to note that the epidermis and the nervous system are both formed from the same layer of the embryo—the epiblast.

## LESSON IV

*MILK, FLOUR, BREAD*

**A. Milk.**—1. Examine a drop of milk with the microscope.

2. Note the specific gravity of fresh milk with the lactometer, and observe that the specific gravity is increased by the removal of the lightest constituent—the cream.

3. The reaction of fresh milk is usually neutral or slightly alkaline.

4. Warm some milk in a test-tube to the temperature of the body, and add a few drops of *rennet*. After standing, a *curd* is formed from the conversion of *caseinogen*, the chief proteid in milk, into *casein*. The casein entangles the fat globules; the liquid residue is termed *whey*. No curd forms if the rennet solution is previously boiled. Heat kills ferment.

5. To another portion of warm milk diluted with water add a few drops of strong acetic acid. A lumpy precipitate of *caseinogen* entangling the fat is formed.

6. Filter off the curd, and in the whey test for *lactose* or *milk sugar* by Trommer's test (see Lesson I.); for *lactalbumin* by boiling, or by Millon's reagent (see Lesson II.); and for earthy (that is, calcium and magnesium) *phosphates* by ammonia, which precipitates these phosphates.

7. *Fat (butter)* may be extracted from the curd by shaking it with ether; on evaporation of the ethereal extract the fat is left behind, forming a greasy stain on paper.

8. *Caseinogen*, like globulins, is precipitated by saturating milk with sodium chloride or magnesium sulphate, but differs from the globulins in not being coagulated by heat. The precipitate produced by saturation with salt floats to the surface with the entangled fat, and the clear salted whey is seen below after an hour or two.

**B. Flour.**—Mix some wheat flour with a little water into a stiff dough. Wrap this up in a piece of muslin and knead under a tap or in a capsule of water. The starch grains come through the holes in the muslin (identify by iodine test), and an elastic sticky mass remains behind. This is a proteid called *gluten* (identify by xanthoproteic or Millon's reaction).

**C. Bread** contains the same constituents as flour except that some of the starch has been converted into dextrin and dextrose during baking (most flours, however, contain a small quantity of sugar). Extract bread with cold water, and test the extract for dextrin (iodine test) and for dextrose (Trommer's test). If hot water is used starch also passes into solution.

## THE PRINCIPAL FOOD-STUFFS

We can now proceed to apply the knowledge we have obtained of the proteids, carbohydrates, and fats to the investigation of some important foods. We do not actually use as food the various organic

proximate principles in the pure condition ; it is necessary that in a suitable diet these should be mixed in certain proportions, and in nature we find them already mixed for us. The chief proximate principles in food are :—

- |                  |   |            |
|------------------|---|------------|
| 1. Proteids      | } | organic.   |
| 2. Carbohydrates |   |            |
| 3. Fats          |   |            |
| 4. Water         |   | inorganic. |
| 5. Salts         |   |            |

In milk and in eggs, which form the exclusive food-stuffs of young animals, all varieties of these proximate principles are present in suitable proportions. Hence they are spoken of as perfect foods. Eggs, though a perfect food for the developing bird, contains too little carbohydrate for a mammal. In most vegetable foods carbohydrates are in excess, while in animal food, like meat, the proteids are predominant. In a suitable diet these should be mixed in suitable proportions, which must vary for herbivorous and carnivorous animals. We must, however, limit ourselves to the omnivorous animal, man.

A healthy and suitable diet must possess the following characters :—

1. It must contain the proper amount and proportion of the various proximate principles. *also*
2. It must be adapted to the climate, age, and sex of the individual and to the amount of work done by him.
3. The food must contain not only the necessary amount of proximate principles, but these must be present in a digestible form. As an instance of this many vegetables (peas, beans, lentils) contain even more proteid than beef and mutton, but are not so nutritious, as they are less digestible, much passing off in the faeces unused.

The nutritive value of a diet depends chiefly on the amount of carbon and nitrogen it contains. A man doing a moderate amount of work will eliminate, chiefly from the lungs in the form of carbonic acid, from 250 to 280 grammes of carbon *per diem*. During the same time he will eliminate, chiefly in the form of urea in the urine, about 15 to 18 grammes of nitrogen. These substances are derived from the metabolism of the tissues, and various forms of energy, work and heat being the chief, are simultaneously liberated. During muscular exercise the output of carbon greatly increases ; the increased excretion of nitrogen is so small as to be negligible. Taking, then, the state of moderate exercise, it is necessary that the waste of the tissues should be replaced by fresh material in the form of food ; and the proportion of carbon to nitrogen should be the same as in the excretions : 250 to 15,

or 16·6 to 1. The proportion of carbon to nitrogen in proteid is, however, 53 to 15, or 8·5 to 1. The extra supply of carbon must come from non-nitrogenous food—viz., fat and carbohydrate.

Moleschott gives the following daily diet :—

Proteid	120	grammes.
Fat	90	"
Carbohydrate	333	"

Ranke's diet closely resembles Moleschott's ; it is—

Proteid	100	grammes.
Fat	100	"
Carbohydrate	250	"

### MILK

**Milk**, which we have seen is an approximately perfect food, is not absolutely so. Bunge has shown it contains too little iron. The microscope reveals that it consists of two parts : a clear fluid and a

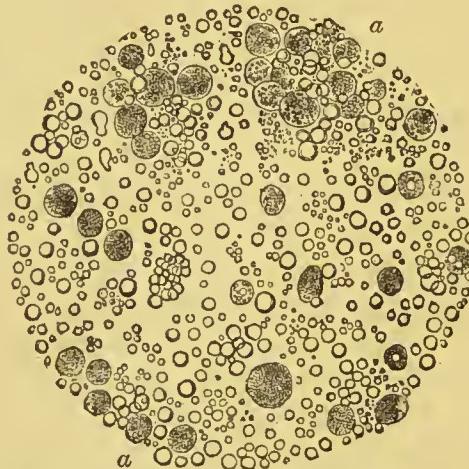


FIG. 10.—Microscopic appearance of milk in the early stage of lactation, showing colostrum corpuscles. (Yeo.)

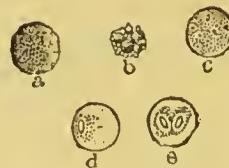


FIG. 11.—a, b, colostrum corpuscles with fine and coarse fat globules respectively; c, d, e, pale cells devoid of fat. (Heidenhain.)

number of minute particles that float in it. These consist of minute oil globules, varying in diameter from 0·0015 to 0·005 millimetre.

The milk secreted during the first few days of lactation is called *colostrum*. It contains very little caseinogen, but large quantities of globulin instead. Microscopically, cells from the acini of the mammary gland are seen, which contain fat globules in their interior : they are called colostrum corpuscles.

**Reaction and Specific Gravity.**—The reaction of fresh cow's milk and of human milk is generally neutral or slightly alkaline. In carnivora the milk is acid. All milk readily turns acid or sour as the

result of fermentative change, part of its lactose being transformed into lactic acid. The specific gravity of milk is usually ascertained with the hydrometer. That of normal cow's milk varies from 1028 to 1034. When the milk is skimmed the specific gravity rises, owing to the removal of the light constituent, the fat, to 1033 to 1037. In all cases the specific gravity of water with which other substances are compared is taken as 1000.

**Composition.**—Frankland gives the following table, contrasting the milk of woman, ass, and cow:—

—	Woman	Ass	Cow
	Per cent.	Per cent.	Per cent.
Proteids (chiefly caseinogen) .	2·7	1·7	4·2
Butter (fat) . : : : :	3·5	1·3	3·8
Lactose . : : : :	5·0	4·5	3·8
Salts . : : : :	0·2	0·5	0·7

Hence, in feeding children on cow's milk, it will be necessary to dilute it, and add sugar to make it approximately equal to natural human milk.

**The Proteids of Milk.**—The principal proteid in milk is called *caseinogen*; this is the one which is coagulated by rennet to form *casein*. Cheese consists of casein with the entangled fat. The other proteid in milk is an albumin. It is present in small quantities only; it differs in some of its properties (specific rotation, coagulation temperature, and solubilities) from serum-albumin; it is called *lact-albumin*.

**The Coagulation of Milk.**—When milk is allowed to stand the chief change that it undergoes is a conversion of part of its lactose into lactic acid. The acid so formed precipitates a portion of the caseinogen. This, however, must not be confounded with the formation of casein from caseinogen. Sometimes milk does undergo true coagulation. This is produced by certain bacterial growths that act like rennet. Rennet, however, is the agent usually employed for this purpose: it is a ferment secreted by the stomach, especially by sucking animals, and is usually obtained from the calf.

The *curd* consists of the casein and entangled fat: the liquid residue called *whey* contains the sugar, salts, and albumin of the milk. There is also a small quantity of a new proteid called *whey-proteid*, which differs from caseinogen by not being convertible into casein. It is produced by the decomposition of the caseinogen molecule.

The curd formed in human milk is more finely divided than that in cow's milk; hence it is more digestible. In feeding children and

invalids on cow's milk, the lumpy condition of the curd may be obviated by the addition of lime water or barley water to the milk.

Caseinogen itself may be precipitated by acids such as acetic acid, or by saturation with neutral salts like sodium chloride. This, however, is not coagulation, but precipitation. The precipitate may be collected and dissolved in lime water; the addition of rennet then produces coagulation in this solution, provided that a sufficient amount of calcium salts is present. We shall find that in blood also the presence of calcium salts is necessary for coagulation. Caseinogen is often compared to alkali-albumin. The latter, however, does not clot with rennet, and is, unlike caseinogen, readily soluble in acids. Caseinogen is not a globulin, though it is like globulins precipitated by neutral salts. It differs from a globulin in not being coagulated by heat. It appears to be a nucleo-albumin; a compound that is of a proteid, with the proteid-like but phosphorus-rich material called nuclein.

**The Fats of Milk.**—The chemical composition of the fat of milk (butter) is very like that of adipose tissue. It consists chiefly of palmitin, stearin, and olein. There are, however, smaller quantities of fats derived from fatty acids lower in the series, especially butyric and caproin. The relation between these varies somewhat, but the proportion is roughly as follows:—Olein,  $\frac{3}{7}$ ; palmitin,  $\frac{1}{3}$ ; stearin,  $\frac{1}{6}$ ; butyric, caproin, and caprylin,  $\frac{1}{4}$ . The old statement that each fat globule is surrounded by a film of caseinogen is not now regarded as true by most authorities. Milk also contains small quantities of lecithin, a phosphorised fat; of cholesterol, an alcohol which resembles fat in its solubilities (see BILE), and a yellow fatty pigment or lipochrome.

**Milk Sugar, or Lactose.**—This is a saccharose ( $C_{12}H_{22}O_{11}$ ). Its general properties have already been described in Lesson I. (p. 12).

The formation of lactic acid from lactose by the activity of certain fungoid growths gives rise to the souring of stale milk. Milk sugar by inverting ferment is changed into dextrose and galactose. When yeast is added, as in the preparation of koumiss, these sugars undergo the alcoholic fermentation.

**The Salts of Milk.**—The chief salt present is calcium phosphate; a small quantity of magnesium phosphate is also present. The other salts are chiefly chlorides of potassium and sodium.

## FLOUR

The best wheat flour is made from the interior of wheat grains, and contains the greater proportion of the starch of the grain and

most of the proteid. Whole flour is made from the whole grain *minus* the husk, and thus contains not only the white interior but also the harder and browner outer portion of the grain. This outer region contains a somewhat larger proportion of the proteids of the grain. Whole flour contains 1 to 2 per cent. more proteid than the best white flour, but it has the disadvantage of being less readily digested. Brown flour contains a certain amount of bran in addition : it is still less digestible, but is useful as a mild laxative, the insoluble cellulose mechanically irritating the intestinal canal as it passes along.

The best flour contains very little sugar. The presence of sugar indicates that germination has commenced in the grains. In the manufacture of malt from barley this is purposely allowed to go on.

When mixed with water, wheat flour forms a sticky adhesive mass called dough. This is due to the formation of gluten, and the forms of grain poor in gluten cannot be made into dough (oats, rice, &c.). Gluten does not exist in the flour as such, but is formed on the addition of water from the pre-existing globulins in the flour (see more fully Lesson XVI.).

The following table contrasts the composition of some of the more important vegetable foods :—

Constituents	Wheat	Barley	Oats	Rice	Lentils	Peas	Potatoes
Water . . . .	13·6	13·8	12·4	13·1	12·5	14·8	76·0
Proteid . . . .	12·4	11·1	10·4	7·9	24·8	23·7	2·0
Fat . . . .	1·4	2·2	5·2	0·9	1·9	1·6	0·2
Starch . . . .	67·9	64·9	57·8	76·5	54·8	49·3	20·6
Cellulose . . . .	2·5	5·3	11·2	0·6	8·6	7·5	0·7
Mineral salts . . . .	1·8	2·7	3·0	1·0	2·4	3·1	1·0

We see from this table—

1. The great quantity of starch always present.
2. The small quantity of fat ; that bread is generally eaten with butter is a popular recognition of this fact.
3. Proteid, except in potatoes, is pretty abundant, and especially so in the pulses (lentils, peas, &c.). The proteid in the pulses is not gluten, but consists of vitellin and other globulin-like substances.

In the mineral matters in vegetables, salts of potassium and magnesium are, as a rule, more abundant than those of sodium and calcium.

## BREAD

**Bread** is made by cooking the dough of wheat flour mixed with yeast, salt, and flavouring materials. A ferment in the flour acts

at the commencement of the baking, when the temperature of the oven is little over that of the body, and forms dextrin and sugar from the starch, and then the alcoholic fermentation, due to the action of the yeast, begins. The bubbles of carbonic acid, burrowing passages through the bread, make it light and spongy. This enables the digestive juices subsequently to soak into it readily and affect all parts of it. In the later stages of baking, the gas and alcohol are expelled from the bread, the yeast is killed, and a crust forms from the drying of the outer portions of the dough.

White bread contains, in 100 parts, 7 of proteid, 55 of carbohydrates, 1 of fat, 2 of salts, and the rest water.

### COOKING OF FOOD

The cooking of foods is a development of civilisation, and much relating to this subject is a matter of education and taste rather than of physiological necessity. Cooking, however, serves many useful ends :—

1. It destroys all parasites and danger of infection. This relates not only to bacterial growths, but also to larger parasites, such as tapeworms and trichina.
2. In the case of vegetable foods it breaks up the starch grains, bursting the cellulose and allowing the digestive juices to come into contact with the granulose.
3. In the case of animal foods it converts the insoluble collagen of the universally distributed connective tissues into the soluble gelatin. By thus loosening the binding material, the more important elements of the food, such as muscular fibres, are rendered accessible to the gastric and other juices. Meat before it is cooked is generally kept a certain length of time to allow *rigor mortis* to pass off.

Of the two chief methods of cooking, roasting and boiling, the former is the more economical, as by its means the meat is first surrounded with a coat of coagulated proteid on its exterior, which keeps in the juices to a great extent, letting little else escape but the dripping (fat). Whereas in boiling, unless both bouillon and bouilli are used, there is considerable waste. Cooking, especially boiling, renders the proteids more insoluble than they are in the raw state; but this is counterbalanced by the other advantages that cooking possesses.

In making beef tea and similar extracts of meat it is necessary that the meat should be placed in cold water, and this is gradually and carefully warmed. In cooking a joint it is usual to put the meat into boiling water at once, so that the outer part is coagulated, and the loss of material minimised.

## LESSON V

*SALIVA*

1. To a little saliva in a test-tube add acetic acid. Mucin is precipitated in stringy flakes.

2. Filter some fresh saliva to separate cells and mucus, and apply the xanthoproteic or Millon's test to the filtrate; the presence of proteid is shown.

3. Put some 0·5-per-cent starch solution into two test-tubes. Add some filtered saliva to one of them, and put both in the water-bath at 40° C. After five minutes remove them and test both fluids with iodine and Trommer's test. The saliva will be found to have converted the starch into dextrin and sugar (maltose).

4. The presence of potassium sulphocyanide (KCNS) in saliva may be shown by the red colour given by a drop of ferric chloride. This colour is discharged by mercuric chloride.

5. The reaction of saliva is alkaline to litmus paper.

The **saliva** is the first digestive juice to come in contact with the food ; it is secreted by three pairs of salivary glands, the parotid, submaxillary and sublingual. The secretions from these differ somewhat in composition, but they are mixed in the mouth, the secretion of the minute mucous glands of the mouth and a certain number of epithelial cells and *débris* being added to it. The so-called 'salivary corpuscles' are derived from the glands themselves.

The secretion of saliva is a reflex action ; the taste or smell of food excites the nerve endings of the afferent nerves (glossopharyngeal and olfactory) : the efferent or secretory nerves are contained in the chorda tympani (a branch of the seventh cranial nerve) which supplies the submaxillary and sublingual, and in a branch of the glossopharyngeal which supplies the parotid. The sympathetic branches which supply the blood vessels with constrictor nerves contain in some animals secretory fibres also.

The parotid gland is called a serous or albuminous gland ; before secretion the cells of the acini are swollen out with granules ; after secretion has occurred the cells shrink, owing to the granules having been shed out to contribute to the secretion (see fig. 12).

The submaxillary and sublingual glands are called mucous glands : their secretion contains mucin. Mucin is absent from parotid saliva.

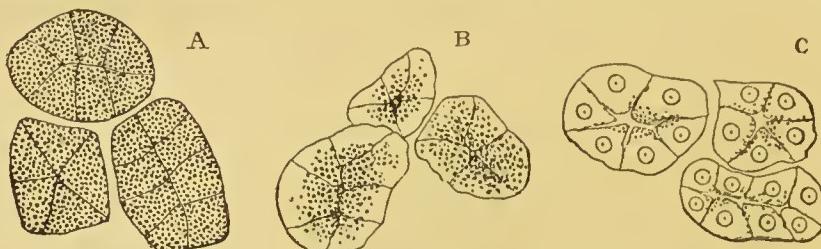


FIG. 12.—Alveoli of serous gland : A, loaded before secretion ; B, after a short period of active secretion ; C, after a prolonged period. (Langley.)

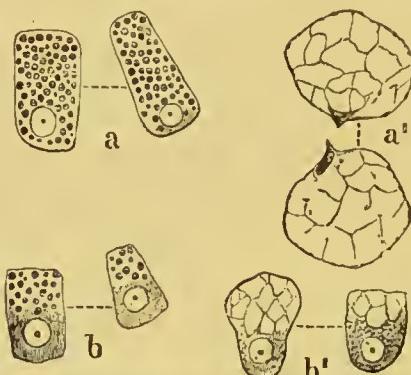


FIG. 13.—Mucous cells from a fresh submaxillary gland of dog : a, loaded with mucinogen granules before secretion ; b, after secretion : the granules are fewer, especially at the attached border of the cell ; a' and b' represent cells in a loaded and discharged condition respectively which have been irrigated with water or dilute acid. The mucous granules are swollen into a transparent mass of mucin traversed by a network of protoplasmic cell-substance. (Foster, after Langley.)

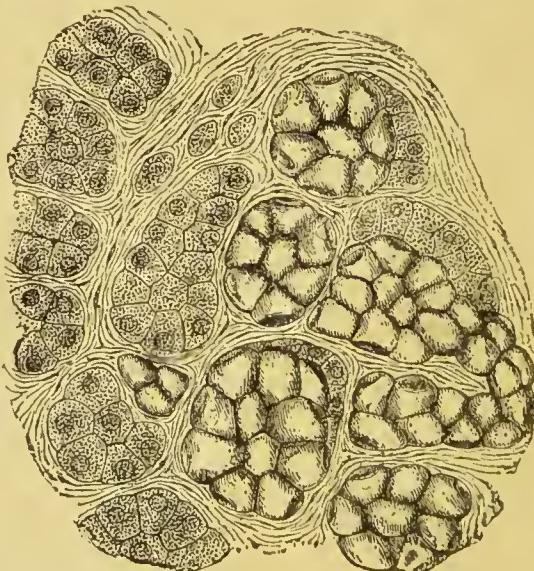


FIG. 14.—Section of part of the human submaxillary gland. (Heidenhain.) To the right is a group of mucous alveoli, to the left a group of serous alveoli.

The granules in the cells are larger than those of the parotid gland. They are composed of mucinogen, the precursor of mucin (see fig. 13).

In a section of a mucous gland prepared in the ordinary way the mucinogen granules are swollen out, and give a highly refracting appearance to the mucous acini (see fig. 14).

### COMPOSITION OF SALIVA

On microscopic examination of mixed saliva a few epithelial scales from the mouth and salivary corpuscles from the salivary glands are seen. The liquid is transparent, slightly opalescent, of slimy consistency, and may contain lumps of nearly pure mucin. On standing it becomes more cloudy owing to the precipitation of calcium carbonate, the carbonic acid, which held it in solution as bicarbonate, escaping.

Of the three forms of saliva which contribute to the mixture found in the mouth the sublingual is richest in solids (2·75 per cent.). The submaxillary saliva comes next (2·1 to 2·5 per cent.). When artificially obtained by stimulation of nerves in the dog the saliva obtained by stimulation of the sympathetic is richer in solids than that obtained by stimulation of the chorda tympani. The parotid saliva is poorest in total solids (0·3 to 0·5 per cent.), and contains no mucin. Mixed saliva contains in man an average of about 0·5 per cent. of solids ; it is alkaline in reaction, due to the salts in it ; and has a specific gravity of 1,002 to 1,006.

The solid constituents dissolved in saliva may be classified thus :—

Organic	<ul style="list-style-type: none"> <li>a. Mucin : this may be precipitated by acetic acid.</li> <li>b. Ptyalin : an amylolytic ferment.</li> <li>c. Proteid : of the nature of a globulin.</li> <li>d. Potassium sulphocyanide.</li> </ul>
Inorganic	<ul style="list-style-type: none"> <li>e. Sodium chloride : the most abundant salt.</li> <li>f. Other salts : sodium carbonate, calcium phosphate and carbonate ; magnesium phosphate ; potassium chloride.</li> </ul>

### THE ACTION OF SALIVA

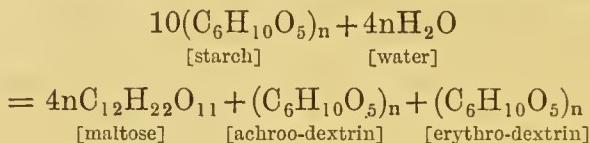
The action of saliva is twofold, physical and chemical.

The physical use of saliva consists in moistening the mucous membrane of the mouth, assisting the solution of soluble substances in the food, and in virtue of its mucin lubricating the bolus of food to facilitate swallowing.

The chemical action of saliva is due to its active principle, ptyalin. This substance belongs to the class of unorganised ferments, and to

that special class of unorganised ferment which are called amyloytic (starch splitting) or diastatic (resembling diastase, the similar ferment in germinating barley and other grains). A general description of ferment will be found at the end of this lesson.

The starch is first split into dextrin and maltose ; the dextrin is subsequently converted into maltose also : this occurs more quickly with erythro-dextrin, which gives a red colour with iodine, than in the other variety of dextrin called achroo-dextrin, which gives no colour with iodine. Brown and Morris give the following equation :—



Ptyalin acts in a similar way, but more slowly on glycogen ; it has no action on cellulose ; hence it is inoperative on uncooked starch grains, in which the cellulose layers are intact.

Ptyalin acts best at about the temperature of the body (35–40°). It acts best in neutral medium ; a small amount of alkali makes but little difference ; a very small amount of acid stops its activity. The conversion of starch into sugar by saliva in the stomach stops after 15–30 minutes, before any free acid appears ; the acid which is just poured out combining with the proteids in the food, and acid proteid retards salivary action. Free hydrochloric acid immediately destroys ptyalin, so that it does not resume work when the semi-digested food once more becomes alkaline in the duodenum.

## FERMENTS

The word **fermentation** was first applied to the change of sugar into alcohol and carbonic acid by means of yeast. The evolution of carbonic acid causes frothing and bubbling ; hence the term 'fermentation.' The agent yeast which produces this is called the ferment. Microscopic investigation shows that yeast is composed of minute rapidly growing unicellular organisms (*torulæ*) belonging to the fungus group of plants.

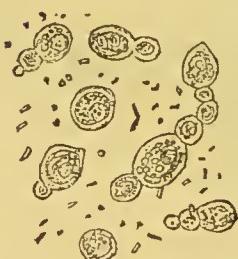


FIG. 15.—Cells of the yeast plant in process of budding, between which are some bacteria. (Yeo's Physiology.)

The souring of milk, the transformation of urea into ammonium carbonate in decomposing urine, and the formation of vinegar (acetic acid) from alcohol are produced by very similar organisms. The complex series of changes known as putrefaction which are accompanied by the formation of malodorous

gases, and which are produced by the various forms of bacteria, also come into the same category.

That the change or fermentation is produced by these organisms is shown by the fact that it occurs only when the organisms are present, and stops when they are removed or killed by a high temperature or

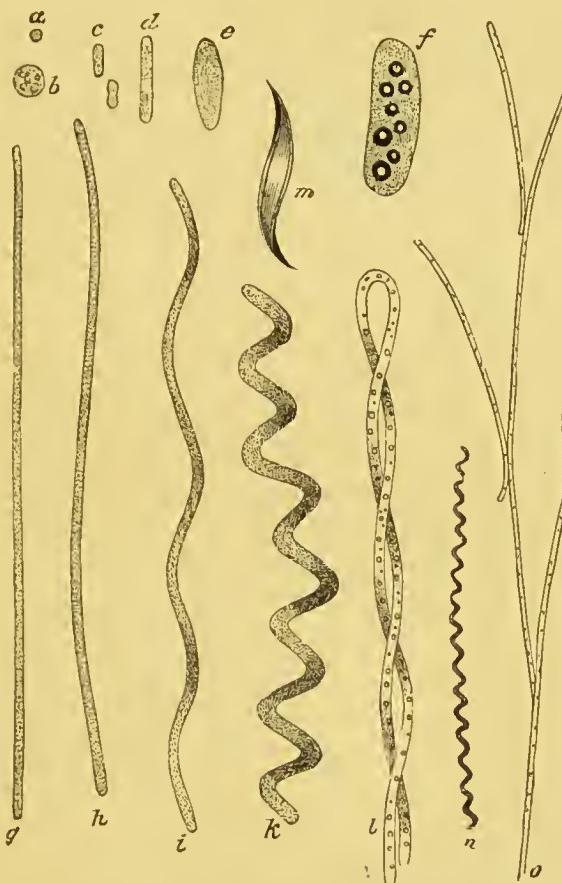


FIG. 16.—Typical forms of Schizomyces (after Zopf) : *a*, Micrococcus ; *b*, macrococcus ; *c*, bacterium ; *d*, bacillus ; *e*, chlostridium ; *f*, Mouas Okunii ; *g*, leptothrix ; *h*, *i*, vibrio ; *k*, spirillum ; *l*, spirulina ; *m*, spironomas ; *n*, spirochaete ; *o*, eladothrix.

by certain substances (carbolic acid, mercuric chloride, &c.) called antiseptics.

The 'germ theory' of disease explains the infectious diseases by considering that the change in the system is of the nature of fermentation, and, like the others we have mentioned, produced by microbes ; the transference of the bacteria or their spores from one person to another constitutes infection. The poisons produced by the growing bacteria appear to be either alkaloidal (ptomaines) or proteid in nature. The existence of poisonous proteids is a very remarkable thing, as no

chemical differences can be shown to exist between them and those which are not poisonous, but which are useful as foods.

There is another class of chemical transformations which differ very considerably from all of these. They, however, resemble these fermentations in the fact that they occur independently of any apparent change in the agents that produce them. The agents that produce them are not living organisms, but chemical substances, the result of the activity of living cells. The change of starch into sugar by the ptyalin of the saliva is an instance.

Ferments may therefore be divided into two classes:—

1. The organised ferments—*torulæ*, bacteria, &c.

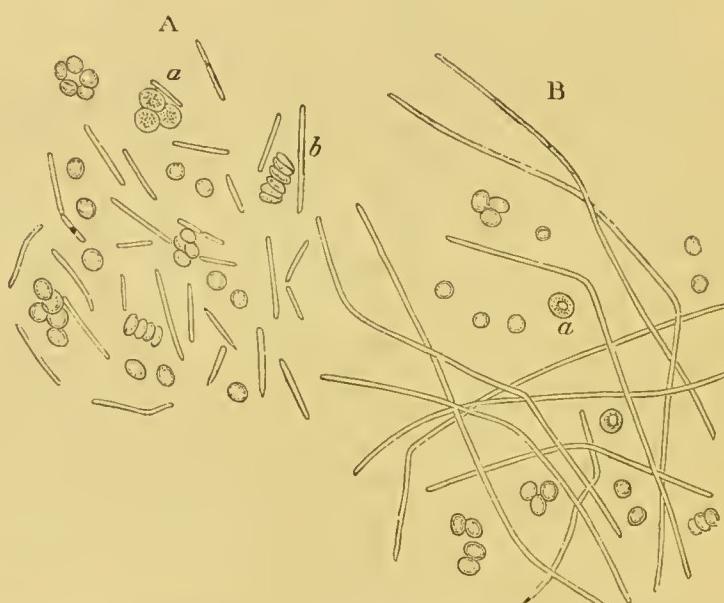


FIG. 17.—*Bacillus anthracis*, the agent that produces anthrax or splenic fever (Koch): A, *Bacilli*, mingled with blood corpuscles from the blood of guinea-pig, some of the bacilli dividing; B, the same after three hours' culture in a drop of aqueous humour. They grow out into long lepto-thrix-like filaments, which subsequently divide up, and spores are developed in the segments.

2. The unorganised ferments or enzymes—like ptyalin.

Each may be again subdivided according to the nature of the chemical change produced.

In digestion, the study of which we are just commencing, it is the unorganised ferments with the action of which we have chiefly to deal.

The unorganised ferments may be classified as follows:—

(a) Amylolytic—those which change amyloses (starch, glycogen) into sugars. Examples: ptyalin, diastase, amylopsin.

(b) Proteolytic—those which change proteids into proteoses and peptones. Examples: pepsin, trypsin.

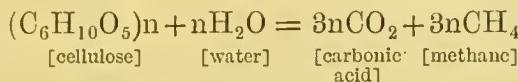
(c) Steatolytic—those which split fats into fatty acids and glycerine. An example, steapsin, is found in pancreatic juice.

(d) Inversive—those which convert saccharoses (cane sugar, maltose, lactose) into glucose. Examples: invertin of intestinal juice and of yeast cells.

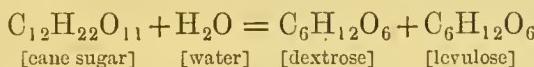
(e) Coagulative—those which convert soluble into insoluble proteids. Examples: rennet, fibrin ferment, myosin ferment.

Most ferment actions are hydrolytic—*i.e.* water is added to the material acted on, which then splits into new materials. This is seen by the following examples:—

1. Conversion of cellulose into carbonic acid and marsh gas (methane) by putrefactive organisms.



2. Inversion of cane sugar by the unorganised ferment invertin:—



A remarkable fact concerning the ferments is that the substances they produce in time put a stop to their activity; thus in the case of the organised ferments the alcohol produced by yeast, the phenol, cresol, &c., produced by putrefactive organisms from proteids, first stop the growth of and ultimately kill the organisms which produce them. In the case of the unorganised ferments, the products of their activity hinder and finally stop their action, but on the removal of these products the ferments resume work.

All ferments act best at a temperature of about 40° C. Their activity is stopped, but the ferments are not destroyed by cold; it is stopped and the ferments killed by too great heat. A certain amount of moisture and oxygen is also necessary; there are, however, certain micro-organisms that act without oxygen, and are called anaërobic in contradistinction to those which require oxygen, and are called aërobic.

The chemical nature of the enzymes or unorganised ferments is very difficult to investigate; they are substances that elude the grasp of the chemist to a great extent. So far, however, research has taught us that the ferments are either proteid in nature or are substances closely allied to the proteids.

## LESSON VI

*PEPTIC DIGESTION*

1. Half fill four test tubes—
  - A with water.
  - B with 0·2 per cent. hydrochloric acid.
  - C with 0·2 per cent. hydrochloric acid.
  - D with solution of white of egg (1 to 10 of water).
2. To A add a few drops of glycerine extract of stomach (this contains pepsin) and a piece of a solid protein like fibrin.  
 To B also add pepsin solution and a piece of fibrin.  
 To C add only a piece of fibrin.  
 To D add a few drops of pepsin solution and fill up the tube with 0·2 per cent. hydrochloric acid.
3. Put all four tubes into the water-bath at 40° C., and observe them from time to time.
  - In A the fibrin remains unaltered.
  - In B it becomes swollen, and gradually dissolves.
  - In C it becomes swollen, but does not dissolve.
4. Examine the solution in test-tube B.
  - (a) Colour some of the liquid with litmus and neutralise with dilute alkali. Acid-albumin, syntoinin, or parapeptone is precipitated.
  - (b) Take another test-tube, and put into it a drop of 1-per-cent. solution of copper sulphate; empty it out so that the merest trace of copper sulphate remains adherent to the wall of the tube; then add the solution from test-tube B and a few drops of strong caustic potash. A pink colour (biuret reaction) is produced. This should be carefully compared with the violet tint given by unaltered albumin.
  - (c) To a third portion of the fluid in test-tube B add a drop of nitric acid; albumoses or propeptides are precipitated. This precipitate dissolves on heating and reappears on cooling.
5. At the end of the lesson these three tests should be repeated with the digested white of egg in test-tube D.

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THE SECRETION OF GASTRIC JUICE

The juice secreted by the glands in the mucous membrane of the stomach varies in composition in the different regions, but the mixed gastric juice, as it may be termed, is a solution of a proteolytic ferment called pepsin in a saline solution, which also contains a little free hydrochloric acid.

The gastric juice can be obtained during the life of an animal by

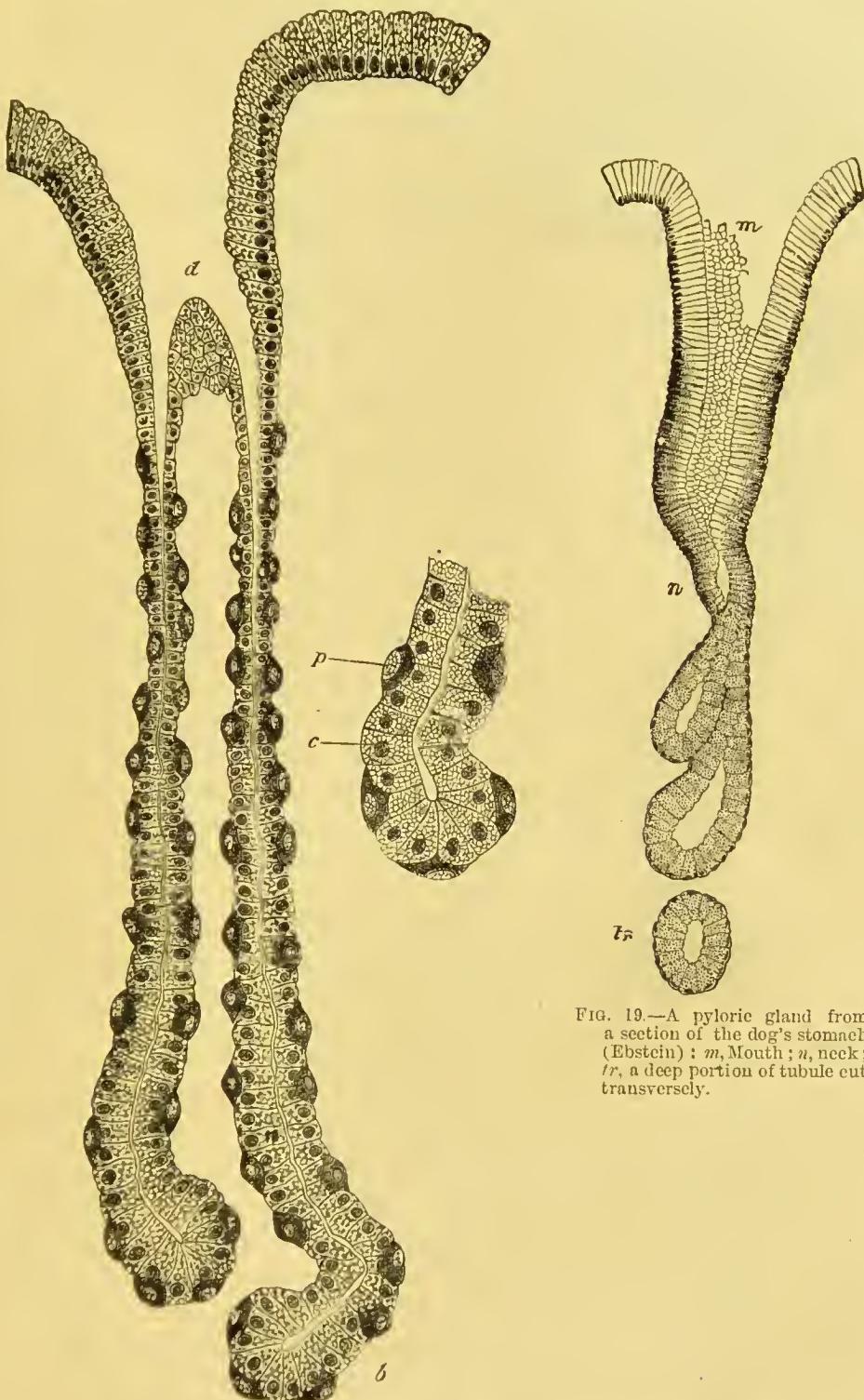


FIG. 18.—A cardine gland from the dog's stomach (Klein) : *d*, Duct or mouth of the gland ; *b*, base or fundus of one of its tubules ; on the right the base of a tubule is more highly magnified ; *c*, central cell ; *p*, parietal cell.

FIG. 19.—A pyloric gland from a section of the dog's stomach (Ebstein) : *m*, Mouth ; *n*, neck ; *tr*, a deep portion of tubule cut transversely.

means of a gastric fistula. Gastric fistulae have also been made in human beings, either by accidental injury or by surgical operations. The most celebrated case is that of Alexis St. Martin, a young Canadian, who received a musket wound in the abdomen in 1822. Observations made on him by Dr. Beaumont formed the starting-point for our correct knowledge of the physiology of the stomach and its secretion.

We now make artificial gastric juice by mixing weak hydrochloric acid (0·2 per cent.) with the glycerine extract of the stomach of a recently killed animal. This artificial juice acts like the normal juice.

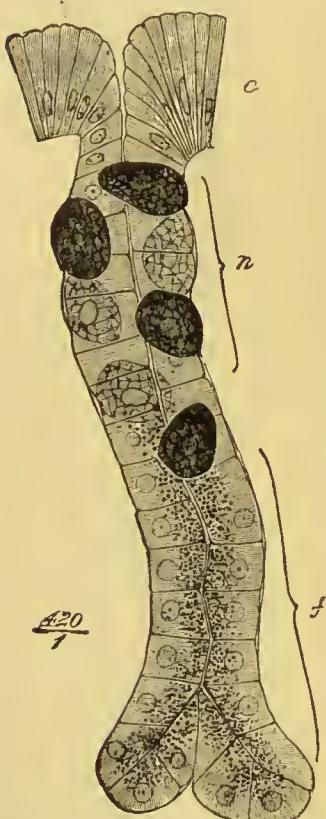


FIG. 20.—A cardiac gland of simple form from the bat's stomach. Osmotic acid preparation (Langley): c, Columnar epithelium of the surface; n, neck of the gland, with central and parietal cells; f, base or fundus, occupied only by principal or central cells, which exhibit the granules accumulated towards the lumen of the gland.

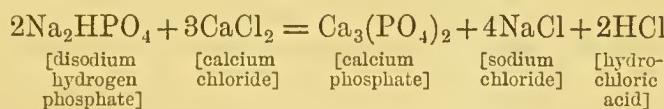
Two kinds of glands are distinguished in the stomach, which differ from each other in their position, in the character of their epithelium, and in their secretion. The *cardiac glands* are those situated in the cardiac part of the stomach: their ducts are short, their tubules long in proportion. The latter are filled with polyhedral cells, only a small lumen being left; they are more closely granular than the corresponding cells in the pyloric glands. They are called *principal* or *central* cells. Between them and the basement membrane of the tubule are other cells which stain readily with aniline dyes. They are called *parietal* or *oxyntic* cells. The *pyloric glands*, in the pyloric part of the stomach, have long ducts and short tubules lined with cubical granular cells. There are no parietal cells.

The *central* cells of the cardiac glands and the cells of the pyloric glands are loaded with granules. During secretion they discharge their granules, those that remain being chiefly situated near the lumen, leaving in each cell a clear outer zone (see fig. 20).

These are the cells that secrete the pepsin. Like secreting cells generally, they select certain materials from the lymph that bathes them; these materials are worked up by the protoplasmic activity of the cells into the secretion, which is then discharged into the lumen of the gland. The most important substance in a digestive secretion is the ferment. In the case of the gastric juice this is pepsin. We can trace an intermediate

step in this process by the presence of the granules. The granules are not, however, composed of pepsin, but of a mother-substance which is readily converted into pepsin. We shall find a similar ferment precursor in the cells of the pancreas, and the term *zymogen* is applied to these ferment precursors. The zymogen in the gastric cells is called *pepsinogen*. The rennet-ferment or rennin that causes the curdling of milk is distinct from pepsin, but is apparently formed by the same cells.

The parietal cells undergo merely a change of size during secretion, being at first somewhat enlarged and after secretion they shrink. They are also called oxytic cells, because it is believed that they secrete the hydrochloric acid of the juice. Heidenhain succeeded in making in one dog a *cul-de-sac* of the fundus, in another of the pyloric region of the stomach; the former secreted a juice containing both acid and pepsin; the latter, parietal cells being absent, secreted a viscid alkaline juice containing pepsin. The formation of a free acid from the alkaline blood and lymph is an important but puzzling problem. There is no doubt that it is formed from the sodium chloride of the blood and lymph, and of the many theories advanced as to how this is done, Maly's is, on the whole, the most satisfactory. He considers that it originates by the interaction of the calcium chloride with the disodium hydrogen phosphate of the blood, thus:—



#### *Composition of Gastric Juice*

The following table gives the percentage composition of the gastric juice of man and the dog:—

Constituents	Human	Dog
Water . . . .	99·44	97·30
Organic substances (chiefly pepsin) . . . .	0·32	1·71
HCl . . . .	0·02	0·30
CaCl <sub>2</sub> . . . .	0·006	0·06
NaCl . . . .	0·14	0·25
KCl . . . .	0·05	0·11
NH <sub>4</sub> Cl . . . .	—	0·05
Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> . . . .		0·17
Mg <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> . . . .		0·02
FePO <sub>4</sub> . . . .	{ 0·01	0·008

One sees from this how much richer in all constituents the gastric juice of the dog is than that of man. Carnivorous animals have

always a more powerful gastric juice than other animals ; they have more work for it to do ; but the great contrast seen in the table is, no doubt, partly due to the fact that the persons from whom it has been possible to collect gastric juice have been invalids. In the foregoing table one also sees the great preponderance of chlorides over other salts ; apportioning the total chlorine to the various metals present, that which remains over must be combined with hydrogen to form the free acid of the juice.

Pepsin stands apart from nearly all other ferments by requiring an acid medium in order that it may act. Probably a compound of the two substances called pepsin-hydrochloric acid is the really active agent. Other acids may take the place of hydrochloric acid, but none act so well. Lactic acid is often found in gastric juice : this appears to be derived by fermentative processes from the food.

### THE ACTION OF GASTRIC JUICE

The principal actions of the gastric juice have been already practically studied ; the action of pepsin in converting the proteids of the food into the diffusible peptones is its chief action. The curdling of milk by rennet will be found described in Lesson IV.

There is still a further action—that is, the gastric juice is anti-septic ; putrefactive processes do not normally occur in the stomach, and the organisms that produce such processes, many of which are swallowed with the food, are in great measure destroyed, and thus the body is protected from them. The acid is the agent in the juice that possesses this power.

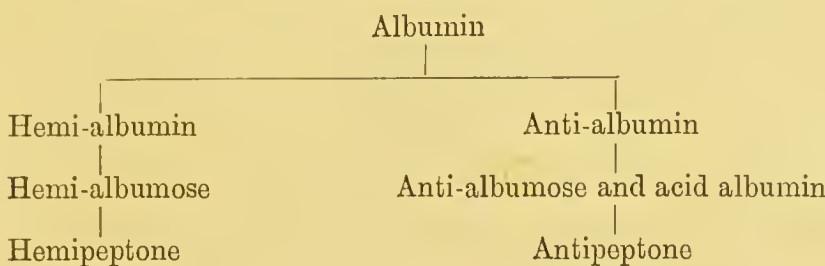
The formation of peptones is a process of hydration ; peptones may be formed by other hydrating agencies like superheated steam and heating with dilute mineral acids. There are certain intermediate steps in this process : the intermediate substances are called pro-peptides or proteoses. The word 'proteose' is the best to employ : it includes the albumoses (from albumin), globuloses (from globulin), vitelloses (from vitellin), &c. Similar substances are also formed from gelatin (gelatinoses) and elastin (elastoses).

Another intermediate step in gastric digestion is called parapeptide : this is acid albumin or syntoinin ; it also, though with some difficulty, is converted into peptide. The products of digestion may be classified in various ways. It will be convenient to take albumin as our example, remembering that globulin, vitellin, and all the other proteids form corresponding products. The products of digestion may be classified, according to their solubilities, as follows :—

1. Parapeptone . . . . . acid albumin.
2. Propeptone  $\left\{ \begin{array}{l} (a) \text{ Proto-albumose} \\ (b) \text{ Hetero-albumose} \\ (c) \text{ Deutero-albumose} \end{array} \right\}$  The primary albumoses, i.e.  
those which are formed first.
3. Peptone.

But a more important classification may be called the physiological classification, and it is the following :—

The albumin molecule may be considered to be made up of two parts called respectively hemi-albumin and anti-albumin. The former yields ultimately hemipeptone, the latter antipeptone. The intermediate albumoses have similar names :—



The hemipeptone differs from the antipeptone in the manner it is affected by the prolonged action of the next digestive juice, the pancreatic secretion ; hemipeptone yields leucine and tyrosine, antipeptone does not. This will be fully discussed in our next lesson.

**Peptones.**—These are the final products of the action of gastric juice on native proteids.

They are soluble in water, are not coagulated by heat, and are not precipitated by nitric acid, copper sulphate, ammonium sulphate, and a number of other precipitants of proteids. They are precipitated but not coagulated by alcohol. They are also precipitated by tannin, picric acid, potassium-mercuric iodide, phospho-molybdic acid, and phospho-tungstic acid.

They gave the biuret reaction (rose-red, with a trace of copper sulphate and caustic potash or soda).

Peptone is readily diffusible through animal membranes. The utility of the formation of diffusible substances during digestion is obvious.

**Proteoses.**—These are the intermediate products in the hydration of native proteids into peptones.

They are not coagulated by heat ; they are precipitated but not coagulated by alcohol ; like peptone they give the biuret reaction. They are precipitated by nitric acid, the precipitate being soluble on

heating, and reappearing when the liquid cools. This last is a distinctive property of proteoses. They are slightly diffusible.

The following table will give us at a glance the chief characters of peptone and proteoses in contrast with those of a native proteid like albumin :—

Variety of proteid	Action of heat	Action of alcohol	Action of nitric acid	Action of ammonium sulphate	Action of copper sulphate and caustic potash	Diffusibility
Albumin	Coagulated	Precipitated, then coagulated	Precipitated in the cold ; not readily soluble on heating.	Precipitated	Violet colour	Nil
Proteoses (albumoses)	Not coagulated	Precipitated, but not coagulated	Precipitated in the cold ; readily soluble on heating ; the precipitate reappears on cooling.	Precipitated	Rose - red colour (biuret reaction)	Slight
Peptones	Not coagulated	Precipitated, but not coagulated	Not precipitated	Not precipitated	Rose - red colour (biuret reaction)	Great

We see that the main action of the gastrice juice is upon the proteids of the food, converting them into more soluble and diffusible products. The fats are not chemically altered in the stomach ; their proteid envelopes are however dissolved, and the solid fats are melted. The carbohydrates are also unaffected.

## LESSON VII

### *PANCREATIC DIGESTION*

1. A 1-per-cent. solution of sodium carbonate, to which a little glycerine extract of pancreas has been added, forms a good artificial pancreatic fluid.
2. Half fill three test-tubes with this solution.
  - A. To this add half its bulk of diluted egg-white (1 in 10).
  - B. To this add a piece of fibrin.
  - C. Boil this; cool; then add fibrin.
3. Put all into the water-bath at 40° C. After half an hour, test A and B for alkali-albumin by neutralisation, for albumoses by nitric acid, and for albumoses and peptone by the biuret reaction.
4. Note in B that the fibrin does not swell up and dissolve, as in gastric digestion, but that it is eaten away from the edges to the interior.
5. In C no digestion occurs, as the ferment have been destroyed by boiling.
6. Take a solution of starch, equal quantities in three test-tubes.
  - D. To this add a few drops of pancreatic extract.
  - E. To this add a few drops of bile.
  - F. To this add both bile and pancreatic extract.
7. Put these into the water-bath, and test small portions of each every half-minute by the iodine reaction. It disappears first in F; then in D; while E undergoes no change. Test D and F for maltose by Trommer's test.
8. Shake up a few drops of olive oil with artificial pancreatic juice. A milky fluid (emulsion) is formed, from which the oil does not readily separate on standing.
9. The foregoing experiments illustrate the action that pancreatic juice has on all three classes of organic food.
  - i. *On Proteids*.—Fibrin, albumin, &c. are converted into proteoses and peptone by the ferment *trypsin* in an alkaline medium.
  - ii. *On Carbohydrates*.—Starch is converted into sugar (maltose) by the ferment *amylopsin*, especially in presence of bile.
  - iii. *On Fats*.—These are emulsified. In the body they are also partly saponified by the ferment *steapsin*; but this cannot be shown with the artificial juice, as steapsin is not soluble in glycerine.

The pancreas is a compound racemose gland, like the parotid. There are, however, histological points of difference between them. Microscopic examination of the gland-cells in different stages of activity reveals changes comparable to those already described in the case of salivary and gastric cells. Granules indicating the presence of a zymogen which is called *trypsinogen* (that is, the precursor of trypsin,

the most important ferment of the pancreatic juice) crowd the cells before secretion: these are discharged during secretion, so that in an animal whose pancreas has been powerfully stimulated to secrete, as by the administration of pilocarpine, the granules are seen only at the free border of the cells (see fig. 21).

As in gastric juice, experiments on the pancreatic secretion are usually performed with an artificial juice, made by mixing a weak

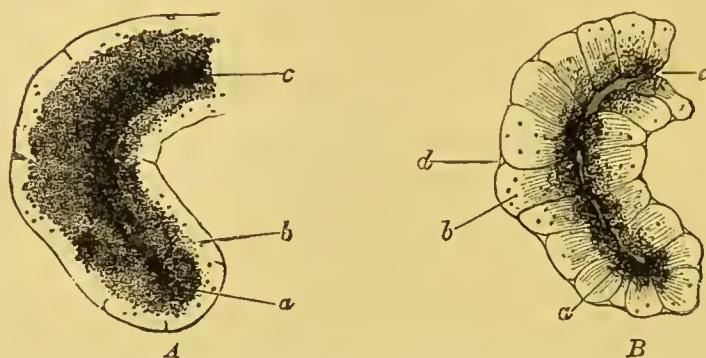


FIG. 21.—Part of an alveolus of the rabbit's pancreas: A, before discharge; B, after.  
(From Foster, after Kühne and Lea.)

alkaline solution (1 per cent. sodium carbonate) with glycerine extract of pancreas.

Quantitative experiments on human pancreatic juice gave the following results:—

Water	97·6	per cent.
Organic solids	1·8	"
Inorganic salts	0·6	"

The organic substances in pancreatic juice are—

(a) Ferments. These are the most important both quantitatively and functionally. They are four in number:—

- i. Trypsin, a proteolytic ferment.
- ii. Amylopsin or pancreatic diastase, an amyloytic ferment.
- iii. Steapsin, a fat-splitting ferment.
- iv. A milk-curdling ferment.

(b) A small amount of proteid matter, coagulable by heat.

(c) Traces of leucine, tyrosine, xanthine, and soaps.

The inorganic substances in pancreatic juice are—

Sodium chloride, which is the most abundant, and smaller quantities of potassium chloride, and phosphates of sodium, calcium, and magnesium. The alkalinity of the juice is due to phosphates and carbonates, especially of sodium.

### ACTION OF PANCREATIC JUICE

The action of pancreatic juice, which is the most powerful and important of all the digestive juices, may be described under the headings of its four ferments.

**1. Action of Trypsin.**—Trypsin acts like pepsin, but with certain differences, which are as follows:—

(a) It acts in an alkaline, pepsin in an acid medium.

(b) It acts more rapidly than pepsin, but the same series of proteoses can be detected as intermediate products in the formation of peptone.

(c) An albuminate of the nature of alkali-albumin is formed in place of the acid-albumin of gastric digestion.

(d) It acts more powerfully on certain albuminoids (such as elastin) which are difficult of digestion in gastric juice.

(e) Acting on solid proteids like fibrin, it eats them away from the surface to the interior; there is no preliminary swelling as in gastric digestion.

(f) Trypsin acts further than pepsin, on prolonged action partly decomposing the *hemipeptone* which has left the stomach into simpler products, of which the most important are leucine and tyrosine. It leaves the *antipeptone* unaffected.

**2. Action of Amylopsin.**—The conversion of starch into maltose is the most powerful and rapid of all the actions of the pancreatic juice. It is much more powerful than saliva, and will act even on unboiled starch. The absence of this ferment in the pancreatic juice of infants is an indication that milk, and not starch, is their natural diet.

**3. Action on Fats.**—The action of pancreatic juice on fats is a double one: it forms an *emulsion*, and it decomposes the fats into fatty acids and glycerin by means of its fat-splitting ferment steapsin. The fatty acids unite with the alkaline bases to form soaps (*saponification*). The chemistry of this is described on p. 17. The fat-splitting power of pancreatic juice cannot be studied with a glycerine extract, as steapsin is not soluble in glycerine: either the fresh juice or a watery extract of pancreas must be used.

The formation of an emulsion may be studied in this way. Shake up olive oil and water together, and allow the mixture to stand; the finely-divided oil globules soon separate from and float on the surface of the water; but if a colloid matter like albumin or gum is first mixed with the water, the oil separates more slowly. A more permanent emulsion is formed by an alkaline fluid, and especially when a small amount of

free fatty acid is being continually liberated ; the acid combines with the alkali to form a soap, which is stated to form a thin layer on the outside of each oil globule, and so prevents them running together again. Pancreatic fluid possesses all the necessary qualifications for forming an emulsion :—

- i. It is alkaline.
- ii. It is viscous from the presence of proteid.
- iii. It has the power of liberating free acids.

**4. Milk-curdling Ferment.**—The addition of pancreatic juice to milk causes clotting ; but this action can hardly ever be called into play, as the milk upon which the juice has to act has been already curdled by the rennin of the stomach.

### INTESTINAL DIGESTION

The pancreatic juice does not act alone on the food in the intestines. There are, in addition, the bile, the succus entericus (secreted by the crypts of Lieberkühn), and bacterial action to be considered.

The bile, as we shall find, has little or no digestive action by itself, but combined with pancreatic juice it assists the latter in all its actions. In our practical exercise we have already seen this is true for the digestion of starch. It is also true for the digestion of proteid, and very markedly so for the digestion of fat. Occlusion of the bileduct by a gall-stone or by inflammation prevents bile entering the duodenum. Under these circumstances the faeces contain a large amount of undigested fat.

The *succus entericus* appears to have to some extent the power of converting starch into sugar ; whether it acts on proteids or fats is very doubtful ; its most important action is due to a ferment it contains called *invertin*, which inverts saccharoses<sup>1</sup>—that is, it converts cane sugar and maltose into glucose.

**Bacterial Action.**—The gastric juice is an antiseptic ; the pancreatic juice is not. A feebly-alkaline fluid like pancreatic juice is just the most suitable medium for bacteria to flourish in. Even in an artificial digestion the fluid is very soon putrid, unless special precautions to exclude or kill bacteria are taken. It is often difficult to say where pancreatic action ends and bacterial action begins, as many of the bacteria that grow in the intestinal contents, having reached that situation in spite of the gastric juice, act in the same way as the juice itself. Some

<sup>1</sup> The original use of the term 'inversion' has been explained on p. 11. It may be extended to include the similar hydrolysis of other saccharoses, although there may be no formation of levorotatory substances.

form sugar from starch, others peptone, leucine, and tyrosine from proteids, while others, again, break up fats. There are, however, certain actions that are entirely due to these putrefactive organisms.

i. On carbohydrates. The most frequent fermentation they set up is the lactic acid fermentation : this may go further and result in the formation of carbonic acid, hydrogen, and butyric acid (see p. 13). Cellulose is broken up into carbonic acid and methane. This is the chief cause of the gases in the intestine, the amount of which is increased by vegetable food.

ii. On fats. In addition to acting like steapsin, lower acids (valeric, butyric, &c.) are produced. The formation of acid products from fats and carbohydrates gives to the intestinal contents in the lower part of the intestine an acid reaction.

iii. On proteids. Fatty acids and amido-acids, especially leucine and tyrosine, are produced ; but these putrefactive organisms have a special action in addition, producing substances having an evil odour, like indole, skatole, and phenol. There are also gaseous products in some cases.

If excessive, putrefactive processes are harmful ; if within normal limits, they are useful, helping the pancreatic juice and, further, preventing the entrance into the body of poisonous products. It is possible that, in digestion, poisonous alkaloids are formed. Certainly this is so in one well-known case. Lecithin, a material contained in small quantities in many foods, and in large quantities in egg-yolk and brain, is broken up by the pancreatic juice into glycerine, phosphoric acid, stearic acid, and an alkaloid called choline. We are, however, protected from the poisonous action of choline by the bacteria, which break it up into carbonic acid, methane, and ammonia.

### LEUCINE AND TYROSINE

These two substances have been frequently mentioned in the preceding pages. As types of the decomposition products of proteids they are important, though probably only small quantities are normally formed during digestion.

They belong to the group of amido-acids. On p. 16 we shall find a list of the fatty acids ; if we replace one of the hydrogen atoms in a fatty acid by amidogen ( $\text{NH}_2$ ), we obtain what is called an amido-acid. Take acetic acid : its formula is  $\text{C}_2\text{H}_4\text{O}_2$  ; replace one H by  $\text{NH}_2$ , and we get  $\text{C}_2\text{H}_3(\text{NH}_2)\text{O}_2$ , which is *amido-acetic acid* or *glycocine*. If we take caproic acid—a term a little higher in the series—its formula is  $\text{C}_6\text{H}_{12}\text{O}_2$  ; amido-caproic acid is  $\text{C}_6\text{H}_{11}(\text{NH}_2)\text{O}_2$ , which is also called

*leucine.* *Tyrosine* is, however, a little more complicated, as it is not only an amido-acid, but also contains an aromatic radicle. Propionic acid has the formula  $C_3H_6O_2$ ; amido-propionic acid is  $C_3H_5(NH_2)O_2$ , and is called alanine. If another H in this is replaced by oxyphenyl

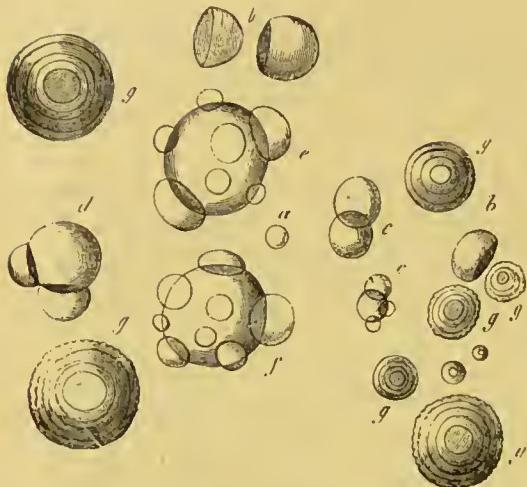


FIG. 22.—Leucine crystals.



FIG. 23.—Tyrosine crystals.

( $C_6H_4\cdot OH$ ), we get  $C_3H_4(C_6H_4\cdot OH)(NH_2)O_2$ , which is oxyphenyl-amido-propionic acid, or tyrosine. Figs. 22 and 23 represent the crystalline forms of leucine and tyrosine.

### EXTIRPATION OF THE PANCREAS

Complete removal of the pancreas in animals and diseases of the pancreas in man produce a condition of diabetes, in addition to the loss of pancreatic action in the intestines. Grafting the pancreas from another animal into the abdomen of the animal from whom the pancreas is removed relieves the diabetic condition.

How the pancreas acts other than in producing the pancreatic juice is not known. It must, however, have other functions related to the general metabolic phenomena of the body, which are disturbed by removal or disease of the gland. This is an illustration of a universal truth—viz. that each part of the body does not merely do its own special work, but is concerned in the great cycle of changes which is called general metabolism. Interference with any organ upsets not only its specific function, but causes disturbances through

the body generally. The interdependence of the circulatory and respiratory systems is a well-known instance. Removal of the thyroid gland upsets the whole body, producing widespread changes known as myxœdema. Removal of the testis produces not only a loss of the spermatic secretion, but changes the whole growth and appearance of the animal. Removal of the greater part of the kidneys produces rapid wasting and the breaking down of the tissues to form an increased quantity of urea. The precise way in which these glands are related to the general body processes is, however, a subject of which we know as yet practically nothing.

## LESSON VIII

*BILE*

1. Ox bile is given round. Observe its colour, taste, smell, and reaction to litmus paper.

2. Acidulate a little bile with acetic acid. A stringy precipitate of a mucinoid substance is obtained. Filter this off and boil the filtrate; no proteid coagulable by heat is present.

3. Prepare some acid-albumin and add a drop or two of bile, or of the above filtrate, or of a solution of bile-salts. The acid-albumin is precipitated. This illustrates the fact that bile-salts precipitate all unpeptonised proteid.

4. *Pettenkofer's Test for Bile Salts.*—To a thin film of bile in a capsule add a drop of solution of cane sugar and a drop of concentrated sulphuric acid. A purple colour is produced. This occurs more quickly on the application of heat.

5. *Gmelin's Test for Bile Pigments.*—On to a little fuming nitric acid in a test-tube pour gently a little bile. Notice the succession of colours—green, blue, red, and yellow—at the junction of the two liquids. This test may also be performed in a capsule. Place a drop of fuming nitric acid in the middle of a thin film of bile; it becomes surrounded by rings of the above-mentioned colours.

6. Examine crystals of cholesterin microscopically.

7. To a few cholesterin crystals add a drop of iodine solution and a drop of concentrated sulphuric acid. A play of blue and red colours is produced.

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Bile is the secretion of the liver which is poured into the duodenum; it has been collected in living animals by means of a biliary fistula; the same operation has occasionally been performed in human beings. After death the gall bladder yields a good supply of bile which is more concentrated than that obtained from a fistula.

Bile is being continuously poured into the intestine, but there is an increased discharge immediately on the arrival of food in the duodenum; there is a second increase in secretion a few hours later.

Though the chief blood supply of the liver is by a vein (the portal vein), the amount of blood in the liver varies with its needs, being increased during the periods of digestion. This is due to the fact that in the area from which the portal vein collects blood—stomach, intestine, spleen, and pancreas—the arterioles are all dilated, and the capillaries are thus gorged with blood. Further, the active peristalsis

of the intestine and the pumping action of the spleen are additional factors in driving more blood onwards to the liver.

The bile being secreted from the portal blood is secreted at much lower pressure than one finds in such glands whose chief blood supply is arterial, such as the salivary glands. Heidenhain found that the pressure in the bile duct of the dog averaged 15 mm. of mercury, which is about double that in the portal vein.

The second increase in the flow of bile—that which occurs some hours after the arrival of the semi-digested food (chyme) in the intestine—appears to be due to the effect of the digestive products carried by the blood to the liver, stimulating the hepatic cells to activity: this is supported by the fact that proteid food increases the quantity of bile secreted, whereas fatty food which is absorbed, not by the portal vein, but by the lacteals, has no such effect.

The chemical processes by which the constituents of the bile are formed are obscure. We, however, know that the biliary pigment is produced by the decomposition of haemoglobin. Bilirubin is, in fact, identical with the iron-free derivative of haemoglobin called haematoidin, which is found in the form of crystals in old blood-clots such as occur in the brain after cerebral haemorrhage (see fig. 24).

An injection of haemoglobin into the portal vein or of substances like water which liberate haemoglobin from the red blood corpuscles produces an increase of bile pigment. If the spleen takes any part in the elaboration of bile pigment, it does not proceed so far as to liberate haemoglobin from the corpuscles. No free haemoglobin is discoverable in the blood plasma in the splenic vein.

The amount of bile secreted is differently estimated by different observers; the amount secreted in man appears to vary from 500 c.c. to 1 litre (1,000 c.c.).

### THE CONSTITUENTS OF BILE

The constituents of the bile are the bile salts proper (taurocholate and glycocholate of soda), the bile pigments (bilirubin, biliverdin), a mucin-like nucleo-albumin, small quantities of fats, soaps, cholesterin, lecithin, urea, and mineral salts, of which sodium chloride and the phosphates of iron, calcium, and magnesium are the most important.

Bile is a yellowish, reddish-brown, or green fluid, according to the



FIG. 24.—Haematoidin crystals.

relative preponderance of its two chief pigments. It has a musk-like odour, a bitter-sweet taste, and a neutral or faintly alkaline reaction.

The specific gravity of human bile from the gall bladder is 1026 to 1032; that from a fistula, 1010 to 1011. The greater concentration of gall bladder bile is partly but not wholly explained by the addition to it from the walls of that cavity of the mucinoid material it secretes.

The amount of solids in bladder bile is from 9 to 14 per cent., in fistula bile from 1·5 to 3 per cent. The following table shows that this low percentage of solids is almost entirely due to want of bile salts. This can be accounted for in the way first suggested by Schiff—that there is normally a bile circulation going on in the body, a large quantity of the bile salts that pass into the intestine being reabsorbed and again secreted. Such a circulation would obviously be impossible in cases where all the bile is discharged to the exterior.

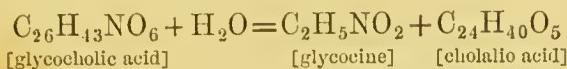
The following table gives some important analyses of human bile :—

Constituents	Fistula bile (healthy woman. Copman and Winston)	Fistula bile (case of cancer. Yeo and Heroun)	Normal bile (Frerichs)
Sodium glycocholate . . .	0·6280	0·165	9·14
Sodium taurocholate . . .		0·055	
Cholesterin, lecithin, fat .	0·0990	0·038	1·18
Mucinoid material . . .	0·1725		2·98
Pigment . . . . .	0·0725	0·148	
Inorganic salts . . . .	0·4510	0·878	0·78
Total solids . . . . .	1·4230	1·284	14·08
Water (by difference). . .	98·5570	98·716	85·92
	100·0000	100·000	100·00

**Bile Mucin.**—There appears to be a small amount of true mucin in the bile, but recent observations have shown that the greater part of the mucin-like substance in the bile is a nucleo-albumin. (For the characters of MUCIN see p. 26; of NUCLEO-ALBUMIN, p. 27.)

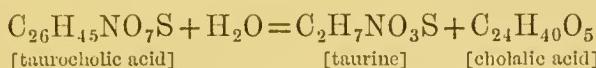
**The Bile Salts.**—The bile contains the sodium salts of complex amido-acids called the bile acids. The two acids most frequently found are glycocholic and taurocholic acids. The former is the more abundant in the bile of man and herbivora; the latter in carnivorous animals, like the dog. The most important difference between the two acids is that taurocholic acid contains sulphur, glycocholic acid not.

**Glycocholic acid** ( $C_{26}H_{43}NO_6$ ) is by the action of dilute acids and alkalis, and also in the intestine, hydrolysed and split into glycocine or amido-acetic acid and cholalic acid.



The glycocholate of soda has the formula  $C_{26}H_{44}NaNO_6$ .

**Taurocholic acid** ( $C_{26}H_{45}NO_7S$ ) similarly splits into taurine or amido-isethionic acid and cholalic acid.



The taurocholate of soda has the formula  $C_{26}H_{44}NaNO_7S$ .

The colour reaction called **Pettenkofer's reaction**, described in the practical exercises at the head of this lesson, is due to the presence of cholalic acid. The sulphuric acid acting on sugar forms a small quantity of a substance called furfuraldehyde, in addition to other products. The furfuraldehyde gives the colour with cholalic acid.

**The Bile Pigments.**—The two chief bile pigments are bilirubin and biliverdin. Bile, which contains chiefly the former (such as dog's bile), is of a golden or orange-yellow colour, while the bile of many herbivora, which contains chiefly biliverdin, is either green or bluish green. Human bile is generally described as containing chiefly bilirubin, but there have been some cases in which biliverdin was in excess. The bile pigments show no absorption bands with the spectroscope : their origin from the blood pigment has already been stated (p. 57).

**Bilirubin** has the formula  $C_{16}H_{18}N_2O_3$  : it is thus an iron-free derivative of haemoglobin. The iron is apparently stored up in the liver cells, perhaps for future use in the manufacture of new haemoglobin. The bile contains only a trace of iron.

**Biliverdin** has the formula  $C_{16}H_{18}N_2O_4$  (*i.e.* one atom of oxygen more than in bilirubin) : it may occur as such in bile ; it may be formed by simply exposing red bile to the oxidising action of the atmosphere ; or it may be formed as in Gmelin's test by the more vigorous oxidation produced by fuming nitric acid.

**Gmelin's test** consists in a play of colours—green, blue, red, and finally yellow, produced by the oxidising action of fuming nitric acid (that is, nitric acid containing nitrous acid in solution). The end or yellow product is called *choletelin*,  $C_{16}H_{18}N_2O_6$ .

**Hydrobilirubin.**—If a solution of bilirubin or biliverdin in dilute alkali is treated with sodium amalgam or allowed to putrefy, a

brownish pigment is formed called hydrobilirubin,  $C_{32}H_{44}N_4O_7$ . With the spectroscope it shows a dark absorption band between *b* and *F*, and a fainter band in the region of the *D* line.

The interest of this substance arises from the fact that many physiologists believe it is identical with *stercobilin*, the pigment of the faeces, and, according to some, with *urobilin*, the chief pigment of the urine. There are, however, certain differences (of solubility, spectroscopic appearances, &c.) which show that the three pigments, though probably closely related, are not absolutely identical.

**Cholesterin.**—This substance is contained not only in bile, but very largely in nervous tissues. Like lecithin, it is an abundant constituent of the white substance of Schwann.

It is found also in blood corpuscles. In bile it is normally present in small quantities only, but it may occur in excess, and form the concretions known as gallstones, which are generally more or less tinged with bilirubin.

Though its solubilities remind one of a fat, cholesterin is not a fat. It is, in fact, chemically speaking, a monatomic alcohol. Its formula is  $C_{26}H_{43}HO$ , or, according to more recent observation,  $C_{27}H_{45}.HO$ .

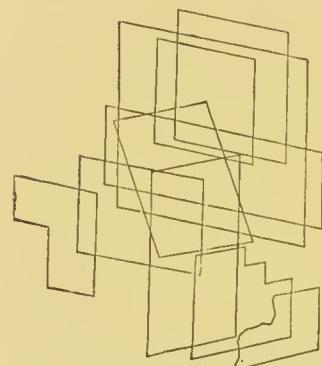


FIG. 25.—Cholesterin crystals.

From alcohol or ether containing water it crystallises in the form of rhombic tables, which contain one molecule of water of crystallisation : these are easily recognised under the microscope (see fig. 25).

It gives the following colour tests :—

1. With iodine and concentrated sulphuric acid the crystals give a play of red, blue, and green.
2. Heated with sulphuric acid and water (5 : 1) the edges of the crystals turn red.
3. A solution of cholesterin in chloroform, shaken with an equal amount of sulphuric acid, turns red, and ultimately purple, the subjacent acid acquiring a green fluorescence.

'The mode of origin of cholesterin in the body has not been clearly made out. Whether it is formed in the tissues generally, in the blood, or in the liver, is not known ; nor has it been determined conclusively that it is derived from albuminous or nervous matter. It is also doubtful if we are to regard it as a waste substance of no use to the body, as its presence in the blood-corpuscles, in nervous matter, in the egg, and in vegetable grains, points to a possible function of a histogenetic or tissue-forming character.' (McKendrick.)

### THE USES OF BILE

One of the most remarkable facts concerning the bile is its apparently small use in the digestion of food. It is doubtless, to a large extent, excretory. Some state it has a slight action on fats and carbohydrates, but it appears to be rather a coadjutor to the pancreatic juice (especially in the digestion of fat) than to have any independent digestive activity (see p. 52).

Bile is said to be a natural antiseptic, lessening the putrefactive processes in the intestine. This is also very doubtful. The bile itself is readily putrescible, and the power it has of diminishing putrescence in the intestine is due chiefly to the fact that by increasing peristalsis it hastens the passage of putrescible matter through the bowel.

When the bile meets the chyme the turbidity of the latter is increased owing to the precipitation of unpeptonised proteid. This is an action due to the bile salts, and it has been surmised that this conversion of the chyme into a more viscid mass is to hinder somewhat its progress through the intestines ; it clings to the intestinal wall, thus allowing absorption to take place. The neutralisation of the acid gastric juice by the bile then allows the alkalinity of the pancreatic juice to have full play.

### THE FATE OF THE BILIARY CONSTITUENTS

We have seen that fistula bile is poor in solids as compared with normal bile, and that this is explained on the supposition that the normal bile circulation is not occurring—the liver cannot excrete what it does not receive back from the intestine. Schiff was the first to show that if the bile is led back into the duodenum, or even if the animal is fed on bile, the percentage of solids in the bile excreted is at once raised. It is on these experiments that the theory of a bile circulation is mainly founded. The bile circulation relates, however, chiefly, if not entirely, to the bile salts : they are found but sparingly in the faeces ; they are only represented to a slight extent in the urine : hence it is calculated that seven-eighths of them are re-absorbed from the intestine. Small quantities of cholic acid, taurine, and glycocine are found in the faeces ; the greater part of these products of the decomposition of the bile salts are taken by the portal vein to the liver, where they are once more synthetised into the bile salts. Some of the taurine is absorbed and excreted as tauro-carbamic acid in the urine. Some of the absorbed glycocine may be excreted as urea. The cholesterol and mucus of the

bile are found in the faeces ; the bile pigment is changed into stercobilin, a substance like hydrobilirubin. Some of the stercobilin may be absorbed, and finally leaves the body as the urinary pigment, urobilin.

### THE FÆCES

The constituents of the faeces may be classified as follows :—

1. Water : in health from 68 to 82 per cent. ; in diarrhoea it is more abundant still.
2. Undigested food ; that is, if food is taken in excess, some escapes the action of the digestive juices. On a moderate diet unaltered proteid is never found.
3. Indigestible constituents of the food : cellulose, keratin, mucin, chlorophyll, gums, resins, cholesterin.
4. Constituents digestible with difficulty ; uncooked starch, tendons, elastin, various phosphates, and other salts of the alkaline earths.
5. Products of decomposition of the food : indole, skatole, phenol, acids such as fatty acids, lactic acid, &c. ; haematin from haemoglobin ; insoluble soaps like those of calcium and magnesium.
6. Bacteria of all sorts and *débris* from the intestinal wall ; cells, nuclei, mucus, &c.
7. Bile residues : mucus, cholesterin, traces of bile acids and their products of decomposition, stercobilin from the bile pigment.<sup>1</sup>

### MECONIUM

Meconium is the name given to the greenish-black contents of the intestine of new-born children. It is chiefly concentrated bile, with *débris* from the intestinal wall. The pigment is a mixture of bilirubin and biliverdin. It is not stercobilin.

### ABSORPTION

Food is digested in order that it may be absorbed. It is absorbed in order that it may be assimilated—that is, become an integral part of the living material of the body.

Having now considered the action of digestive juices, we can study the absorption which follows. In the mouth and oesophagus the thickness of the epithelium and the quick passage of the food through these parts reduce absorption to a minimum. Absorption takes place rapidly in the stomach : the small intestine with its folds and villi to increase its surface is, however, the great place for absorption ; and although the villi are absent from the large intestine, absorption occurs there also, but to a less extent.

<sup>1</sup> Stercobilin may originate also from the haematin of the food. (MacMunn.)

Foods such as water and soluble salts like sodium chloride are absorbed unchanged. The organic foods, however, are considerably changed, colloid materials like starch and proteid being converted respectively into the diffusible materials sugar and peptone.

There are two channels of absorption, the blood vessels (portal capillaries) and the lymphatic vessels or lacteals.

Absorption, however, is no mere physical process of diffusion and filtration. We must also take into account the fact that the cells through which the absorbed substances pass are living, and in virtue of their vital activity not only select materials for absorption, but also change those substances while in contact with them. These cells are of two kinds—(1) the columnar epithelium that covers the surface ; and (2) the lymph cells in the lymphoid tissue beneath. It is now generally accepted that of the two the former, the columnar epithelium, is the more important.

**Absorption of Carbohydrates.**—Though the sugar formed from starch by ptyalin and amylopsin is maltose, that found in the blood is glucose. Under normal circumstances little if any is absorbed by the lacteals. The glucose is formed from the maltose by the succus entericus, and perhaps also by the vital action of the epithelial cells. Cane sugar and milk sugar are also converted into glucose before absorption.

Glucose is taken to the liver, and there stored up in the form of glycogen—a reserve store of carbohydrate material for the future needs of the body. Glycogen, however, is found in animals who take no carbohydrate food. It must, then, be formed by the protoplasmic activity of the liver cells from their proteid constituents. The carbohydrate store apparently leaves the liver in the blood of the hepatic vein as glucose (dextrose) once more.

**Absorption of Proteids.**—A certain amount of soluble proteid is absorbed unchanged. Thus, after taking a large number of eggs, egg albumin is found in the urine. Patients fed *per rectum* derive nourishment from proteid food, though proteolytic ferments are not present in this part of the intestine.

Most proteid, however, is normally absorbed as peptone and proteose (albumose). Peptones and proteoses are absent from the blood under all circumstances, even from the portal blood during the most active digestion. In other words, during absorption the epithelial cells change the products of proteolysis (peptones and proteoses) back once more into native proteids (albumin and globulin).

The greater part of the proteid absorbed passes into the blood ; a little into the lymph vessels also ; but this undergoes the same change.

When peptone (using the word to include the proteoses also) is

injected into the blood stream, poisonous effects are produced, the coagulability of the blood is lessened, the blood pressure falls, secretion ceases, and in the dog 0·3 grammes of 'peptone' per kilogramme of body weight is sufficient to kill the animal.

The epithelial cells of the alimentary canal thus protect us from

those poisonous effects by converting the harmful peptone into the useful albumin.

#### Absorption of Fats.—

The fats undergo in the intestine two changes : one a physical change (emulsification), the other a chemical change (saponification). The lymphatic vessels are the great channels for fat absorption, and their name, lacteals, is derived from the milk-like appearance of their contents (chyle) during the absorption of fat.

The way in which the minute fat globules pass from the intestine into the lacteals has been the subject of much controversy. The course they take may be studied by killing animals at varying periods after a meal of fat, and making osmic acid microscopic preparations of the villi. Figs. 26 and 27 illustrate the appearances observed.

The columnar epithelium cells become first filled with fatty globules of varying size, which

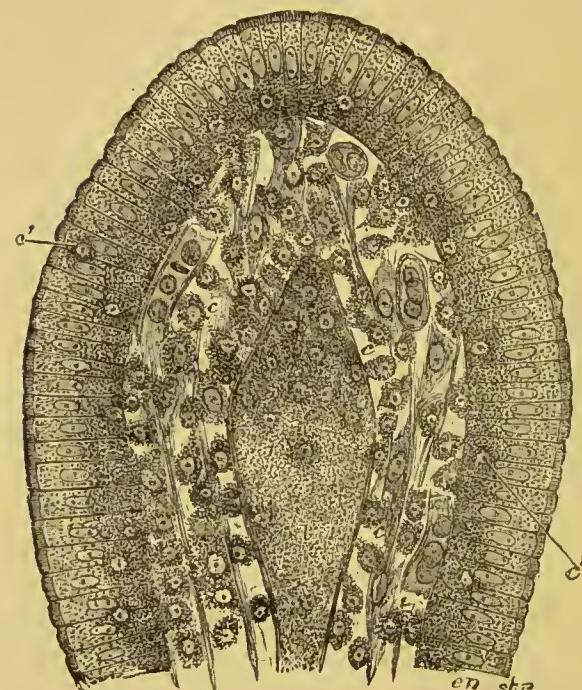


FIG. 26.—Section of the villus of a rat killed during fat absorption (E. A. Schäfer) : *ep*, epithelium ; *str*, striated border ; *c*, lymph cells ; *c'*, lymph cells in the epithelium ; *l*, central lacteal containing disintegrating lymph cells.

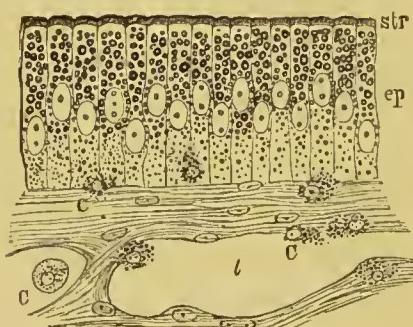


FIG. 27.—Mucous membrane of frog's intestine during fat absorption (E. A. Schäfer) : *ep*, epithelium ; *str*, striated border ; *c*, lymph cells ; *l*, lacteal.

are generally larger near the free border. The globules pass down the cells, the larger ones breaking up into smaller ones during the journey ; they are then transferred to the amoeboid cells of the

lymphoid tissue beneath: these ultimately penetrate into the central lacteal, where they either disintegrate or discharge their cargo into the lymph stream. The globules are by this time divided into immeasurably small ones, the molecular basis of chyle. The chyle enters the blood stream by the thoracic duct, and after an abundant fatty meal the blood plasma is quite milky; the fat droplets are so small that they circulate without hindrance through the capillaries. The fat in the blood after a meal is eventually stored up in the connective tissue cells of adipose tissue. It must, however, be borne in mind that the fat of the body is not exclusively derived from the fat of the food, but it may originate also both from proteid and from carbohydrate.

The great difficulty in fat absorption is how the fat first gets into the columnar epithelium: these cells will not take up other particles, and it appears certain that the epithelial cells do not in the higher animals protrude pseudopodia from their borders. This, however, does occur in the endoderm of some of the lower invertebrates.

Recent research has shown that particles may be present in the epithelium cells while no fat is being absorbed. These particles are apparently protoplasmic in nature, as they stain with reagents that stain protoplasmic granules; they however also stain darkly with osmic acid, and so are apt to be mistaken for fat. It also appears that the epithelial cells have the power of forming fat out of the fatty acids and glycerine into which they have been broken up in the intestine. Munk, who has performed a large number of experiments on the subject, considers that the splitting of fats into glycerine and fatty acids occurs to a much greater extent than is generally supposed; that these substances being soluble pass readily into the epithelium cells; and that these cells perform the synthetic act of building them into fat once more, the fat so formed appearing in the form of small globules, surrounding or becoming mixed with the protoplasmic granules that are ordinarily present.<sup>1</sup> Another remarkable fact recently made out is that after feeding an animal on fatty acids the chyle contains fat. The necessary glycerine must have been formed by protoplasmic activity during absorption.

<sup>1</sup> Munk's views require confirmation before they can be fully accepted. One objection to them is that the largest fat globules are seen near the free border of the cell, whereas if synthesis were occurring one would expect to find the smallest globules in this situation.

## LESSON IX

## THE BLOOD

## Blood Plasma

1. The coagulation of the blood has been prevented in specimen A by the addition of neutral salt (an equal volume of saturated sodium-sulphate solution, or a quarter of its volume of saturated magnesium-sulphate solution). The corpuscles have settled, and the supernatant salted plasma siphoned off.

2. The coagulation of the blood in specimen B has been prevented by the addition of 0·2 per cent. of potassium oxalate in normal saline solution. The oxalated (decalcified) plasma has been siphoned off.

3. Put a small quantity of A into three test-tubes and dilute each with about ten times its volume of liquid :

A 1. With distilled water.

A 2. With solution of fibrin ferment containing a little calcium chloride.

A 3. With the same.

4. Put A 1 and A 2 into the water-bath at 40° C.; leave A 3 at the temperature of the air. A 1 coagulates slowly or not at all; A 2 coagulates rapidly; A 3 coagulates less rapidly than A 2.

5. Add to some of plasma B a few drops of dilute (2 per cent.) calcium-chloride solution : it coagulates, and more quickly, if the temperature is 40° C.

## Hæmoglobin

6. Direct the spectroscope to the window and carefully focus Fraunhofer's lines. Note especially D in the yellow, and E, the next well-marked line, in the green.

7. Direct the spectroscope to a gas flame: these lines are absent. Place a little sodium chloride in the flame. Notice the bright yellow line in the position of the D line.

8. Take a series of six test-tubes of about equal size. Fill the first with diluted defibrinated ox-blood (1 part of blood to 31 of water); then fill the second tube with the same mixture diluted with an equal bulk of water (1 in 64); half fill the third tube with this and fill up the tube with an equal bulk of water (1 in 128), and so on. The sixth tube will contain 1 part of blood to 1,024 of water, and will be nearly colourless.

9. Into another series of six test-tubes put a few drops of ammonium sulphide; then pour in some of the contents of each of the first series and warm *very* gently.

10. Examine the tubes with the spectroscope and map out on a chart the typical absorption bands of oxyhæmoglobin in the first series, and of (reduced) hæmoglobin in the second series. Notice that in the more dilute specimens of hæmoglobin the bands are no longer seen, whereas those of oxyhæmoglobin in specimens similarly diluted are still visible.

11. Take a tube which shows the single band of reduced hæmoglobin and shake it with the air; the bright red colour returns to it, and it shows spectroscopically the two bands of oxyhæmoglobin.

12. Mix a drop of defibrinated rat's blood on a slide with a drop of water, or mount it in a drop of Canada balsam. Examine the crystals of oxyhaemoglobin as they form.

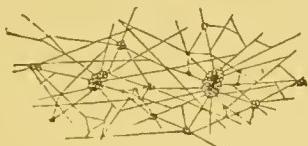
13. Smear a little blood, obtained by pricking the finger, on a slide and allow it to dry; cover, and run glacial acetic acid under the cover glass, and boil. Examine microscopically for the dark brown crystals of haemin.

### COAGULATION OF BLOOD

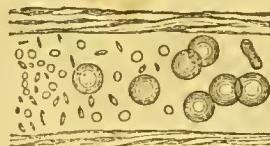
Microscopic investigation of vertebrate blood shows that it consists of a fluid which holds in suspension large numbers of solid bodies—the red and the white corpuscles and the blood tablets.

After blood is shed it rapidly becomes viscous and then sets into a solid red jelly. The jelly soon contracts and squeezes out a straw-coloured fluid called the serum, in which the shrunken clot ultimately floats.

With the microscope filaments of fibrin are seen forming a network throughout the plasma, many radiating from small clumps of blood tablets. It is the formation of fibrin which is the essential act of



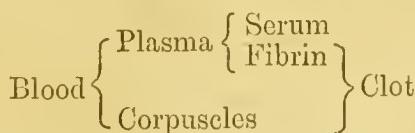
A



B

FIG. 28.—Fibrin filaments and blood tablets. A, network of fibrin shown after washing away the corpuscles from a preparation of blood that has been allowed to clot. Many of the filaments radiate from small clumps of blood tablets; B (from Osler), blood corpuscles and blood tablets within a small vein.

coagulation : this entangles the corpuscles and forms the clot. Fibrin is formed from the plasma, and may be obtained free from corpuscles when blood plasma is allowed to clot, the corpuscles having previously been removed. It may be also obtained from blood by whipping it with a bunch of twigs ; the fibrin adheres to the twigs and entangles but few corpuscles. These may be removed by washing with water. Serum is plasma *minus* fibrin. The relation of plasma, serum, and clot can be seen at a glance in the following scheme of the constituents of the blood :—



It may be roughly stated that in 100 parts by weight of blood 60–65 parts consist of plasma and 35–40 of corpuscles.

The *buffy coat* is seen when blood coagulates slowly, as in horse's blood. The red corpuscles sink more rapidly than the white, and the upper stratum of the clot (buffy coat) consists mainly of fibrin and white corpuscles.

Coagulation is hastened by—

1. A temperature a little over that of the body.
2. Contact with foreign matter.
3. Agitation.
4. Addition of calcium salts.

Coagulation is hindered or prevented by—

1. A low temperature. In a vessel cooled by ice, coagulation may be prevented for an hour or more.

2. The addition of a large quantity of neutral salts like sodium sulphate or magnesium sulphate.

3. Contact with the living vascular walls.

4. Contact with oil.

5. Addition of oxalates : these precipitate the calcium necessary for coagulation as insoluble calcium oxalate.

6. Injection of commercial peptone (which consists chiefly of albumoses) into the circulation of the living animal. The albumose probably acts also by combining with the calcium salts of the blood.

7. Addition of leech extract. This acts in virtue of an albumose it contains.

The theory generally received which accounts best for the coagulation of the blood is that of Hammarsten, and it may be briefly stated as follows :—

*When blood is within the vessels one of the constituents of the plasma, a proteid of the globulin class called fibrinogen, exists in a soluble form.*

*When the blood is shed the fibrinogen molecule is split into two parts : one part is a globulin, which remains in solution, the other is the insoluble material fibrin.*

*This change is brought about by the activity of a special unorganised ferment called the fibrin ferment.*

*This ferment does not exist in healthy blood contained in healthy blood vessels, but is one of the products of the disintegration of the white corpuscles and blood tablets that occurs when the blood leaves the vessels or comes into contact with foreign matter.*

To this it may be added, as the result of recent research, that a soluble calcium salt is essential for the effective action of the ferment ; that probably the fibrin ferment is a proteid allied to the nucleo-albumin class. That other nucleo-albumins (Wooldridge's tissue-

fibrinogens) obtained from most of the cellular organs of the body produce intravascular clotting when injected into the circulation of a living animal.

### THE PLASMA AND SERUM

The liquid in which the corpuscles float may be obtained by employing one or other of the methods already described for preventing the blood from coagulating. The corpuscles being heavy sink, and the supernatant plasma can then be removed by a pipette or siphon, or more thoroughly by the use of a centrifugal machine (see fig. 60).

On counteracting the influence which has prevented the blood from coagulating, the plasma then itself coagulates. Thus plasma obtained by the use of cold clots on warming gently; plasma which has been decalcified by the action of a soluble oxalate clots on the addition of a calcium salt; plasma obtained by the use of a strong solution of salt coagulates when this is diluted by the addition of water, the addition of fibrin ferment being necessary in most cases; where coagulation occurs without the addition of fibrin ferment, no doubt some is already present from the partial disintegration of the corpuscles which has already occurred. Pericardial and hydrocele fluids resemble pure plasma very closely in composition. As a rule, however, they contain few or no white corpuscles, and so do not clot spontaneously, but after the addition of fibrin ferment or liquids like serum that contain fibrin ferment they always yield fibrin.

Pure plasma may be obtained from horse's veins by what is known as the 'living test tube' experiment. If the jugular vein is ligatured in two places, so as to include a quantity of blood within it, then removed from the animal and hung in a cool place, the blood will not coagulate for many hours. The corpuscles settle, and the supernatant plasma can be removed with a pipette.

The plasma is alkaline, yellowish in tint, and its specific gravity is about 1026 to 1029.

Its chief constituents may be enumerated as follows:—

1000 parts of plasma contain—

Water	.	.	.	.	.	.	902·90
Solids	:	.	.	.	.	.	97·10
Proteids: 1, yield of fibrin	.	.	.	.	.	.	4·05
2, other proteids	.	.	.	.	.	.	78·84
Extractives (including fat)	.	.	.	.	.	.	5·66
Inorganic salts	.	.	.	.	.	.	8·55

In round numbers plasma contains 10 per cent. of solids, of which 8 per cent. are proteid in nature.

Serum contains the same three classes of constituents—proteids, extractives, and salts. The extractives and salts are the same in the two liquids. The proteids are different, as is shown in the following table :—

<i>Proteids of Plasma</i>	<i>Proteids of Serum</i>
Fibrinogen	Serum globulin
Serum globulin	Serum albumin
Serum albumin	Fibrin ferment

The gases of the plasma and serum are small quantities of oxygen, nitrogen, and carbonic acid. The greater part of the oxygen of the blood is combined in the red corpuscles with haemoglobin; the carbonic acid is chiefly combined as carbonates.

We may now consider one by one the various constituents of the plasma and serum.

**A. Proteids.—Fibrinogen.**—This is the substance acted on by fibrin ferment. It yields, under this action, an insoluble product called fibrin, and a soluble proteid of the globulin class.

Fibrinogen itself is a globulin. It differs from serum globulin, and may be separated from it by the fact that half-saturation with sodium chloride precipitates it. It coagulates by heat at the low temperature of 56° C.

*Serum globulin and serum albumin.*—The properties of these substances have been already considered (see Lesson II.).

*Fibrin ferment.*—Schmidt's method of preparing it is to take serum and add excess of alcohol. This precipitates all the proteids, fibrin ferment included. After some weeks the alcohol is poured off; the serum-globulin and serum-albumin have been by this means rendered insoluble in water; an aqueous extract is, however, found to contain fibrin ferment, which is not so easily coagulated by alcohol as the other proteids.

**B. Extractives.**—These are non-nitrogenous and nitrogenous. The non-nitrogenous are fats, soaps, cholesterin, and sugar; the nitrogenous are urea (0·02 to 0·04 per cent.), and still smaller quantities of uric acid, creatine, xanthine, and hypoxanthine.

**C. Salts.**—The most abundant salt is sodium chloride: it constitutes between 60 and 90 per cent. of the total mineral matter. Potassium chloride is present in much smaller amount. It constitutes about 4 per cent. of the total ash. The other salts are phosphates and sulphates.

Schmidt gives the following table :—

1000 parts of plasma yield—

Mineral matter . . . . .	8.550
Chlorine . . . . .	3.640
SO <sub>3</sub> . . . . .	0.115
P <sub>2</sub> O <sub>5</sub> . . . . .	0.191
Potassium . . . . .	0.323
Sodium . . . . .	3.341
Calcium phosphate . . . . .	0.311
Magnesium phosphate . . . . .	0.222

### THE WHITE BLOOD CORPUSCLES

Our chemical knowledge of the white corpuscles is small. Their nucleus consists of nuclein, their cell-protoplasm yields proteids belonging to the globulin and nucleo-albumin groups. The nucleo-albumin obtained from them is probably the same thing as fibrin-ferment, or it may be the zymogen of that ferment, the addition of a calcium salt converting it into the ferment. The protoplasm of these cells often contains small quantities of fat and glycogen.

### THE RED BLOOD CORPUSCLES

The red blood corpuscles are much more numerous than the white, averaging in man 5,000,000 per cubic millimetre, or 400 to 500 red to each white corpuscle. The method of enumeration of the corpuscles is described in the Appendix.

They vary in size and structure in different groups of vertebrates. In mammals they are biconcave (except in the camel tribe, where they are biconvex) non-nucleated discs, in man averaging  $\frac{1}{3200}$  inch in diameter; during foetal life nucleated red corpuscles are, however, found. In birds, reptiles, amphibians, and fishes they are biconvex oval discs with a nucleus: they are largest in the amphibia.

Water causes the corpuscles to swell up, and dissolves out the red pigment (oxyhaemoglobin), leaving a globular colourless stroma. Salt solution causes the corpuscles to shrink: they become crenated or wrinkled. The action of water and salt solution suggests the existence of a membrane on the surface of the corpuscles through which osmosis takes place, but the existence of such a membrane is still a matter of discussion. If there is no actual membrane, the outer denser portion of the stroma plays the rôle of one during osmotic phenomena. Dilute alkalis (0.2 per cent. potash) dissolve the corpuscles. Dilute acids (1 per cent. acetic

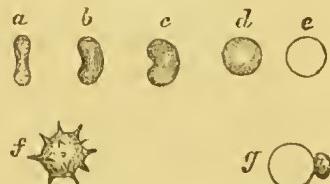


FIG. 29.—a-e, successive effects of water on a red blood corpuscle; f, a red corpuscle crenated by salt solution; g, action of tamin on a red corpuscle.

Dilute alkalis (0.2 per cent. potash) dissolve the corpuscles. Dilute acids (1 per cent. acetic

acid) act like water, and in nucleated corpuscles render the nucleus distinct. *Tannic acid* causes a discharge of haemoglobin from the stroma, but this is immediately altered and precipitated. It remains adherent to the stroma as a brown globule, consisting probably of haematin. *Boracic acid* acts similarly, but in nucleated red corpuscles the pigment collects chiefly round the nucleus, which may then be extruded from the corpuscles.

**Composition.**—1000 parts of red corpuscles contain—

Water . . . . .	688	parts
Solids { organic . . . . .	303·88	"
\ inorganic . . . . .	8·12	"

100 parts of dried corpuscles contain—

Proteid . . . . .	5	to 12	parts
Hæmoglobin . . . . .	86	"	94 "
Lecithin . . . . .	1·8		"
Cholesterin . . . . .	0·1		"

The proteid present appears to be identical with the cell-globulin (nucleo-albumin ?) of white corpuscles. The mineral matter consists chiefly of chlorides of potassium and sodium, and phosphates of calcium and magnesium. In man potassium chloride is more abundant than sodium chloride; this, however, does not hold good for all animals.

Oxygen is contained in combination with the hæmoglobin to form oxyhæmoglobin. The corpuscles also contain a certain amount of carbonic acid (see RESPIRATION, at the end of this lesson).

**Hæmoglobin and Oxyhæmoglobin.**—The pigment is by far the most abundant and important of the constituents of the red corpuscles. It is a substance which gives the reactions of a proteid, but differs from other proteids in containing the element iron, and in being crystallisable.<sup>1</sup>

<sup>1</sup> Hæmoglobin and oxyhæmoglobin are both crystallisable; like other proteids they have enormously large molecules, and so are indissoluble. Though crystalline they are therefore not crystalloid in Graham's sense of that term (see p. 20). Blood pigment is, however, not the only crystallisable proteid. Long ago crystals of proteid (globulin or vitellin) were observed in the aleurone grains of many seeds, and in the similar proteid occurring in the egg-yolk of some fishes and amphibians. By appropriate methods these have been separated and recrystallised. The crystals do not appear to be pure proteid, but compounds with some inorganic substance like lime or magnesia. Further egg-albumin itself has been crystallised. If a solution of white of egg is diluted with half its volume of saturated solution of ammonium sulphate, the globulin present is precipitated, and is removed by filtration. The filtrate is now allowed to remain some days at the temperature of the air, and as it becomes more concentrated from evaporation, minute spheroidal globules of varying size, and finally minute needles, either aggregated or separate, make their appearance. Whether these are pure egg-albumin or compounds of egg-albumin with the ammonium sulphate is at present a matter of dispute.

It exists in the blood in two conditions: in arterial blood it is combined loosely with oxygen, is of a bright red colour, and is called oxyhaemoglobin; the other condition is the deoxygenated or reduced haemoglobin (better called simply haemoglobin). This is found in the blood after asphyxia. It also occurs in all venous blood—that is, blood which is returning to the heart after it has supplied the tissues with oxygen. Venous blood, however, always contains a considerable quantity of oxyhaemoglobin also. Haemoglobin is the oxygen-carrier of the body, and it may be called a respiratory pigment.

Crystals of oxyhaemoglobin may be obtained with readiness from the blood of such animals as the rat, guinea-pig, or dog; with difficulty from other animals such as man, ape, and most of the common mammals. The following methods are the best:—

1. Mix a drop of defibrinated blood of the rat on a slide with a drop of water; put on a cover glass; in a few minutes the corpuscles are rendered colourless, and then the oxyhaemoglobin crystallises out from the solution so formed.

2. Microscopical preparations may also be made by Stein's method, which consists in using Canada balsam instead of water in the above experiment.

3. On a larger scale the crystals may be obtained by mixing the blood with one-sixteenth of its volume of ether; the corpuscles dissolve and the blood assumes a *laky* appearance. After a period, varying from a few minutes to days, abundant crystals are deposited.

The accompanying figures represent the form of the crystals so obtained.

In nearly all animals the crystals are rhombic prisms; in the guinea-pig they are rhombic tetrahedra (four-sided pyramids); in the squirrel, hexagonal plates; in the hamster, rhombohedra and hexagonal plates.

The crystals also contain a varying amount of water of crystallisation: this may in part explain their different crystalline form and solubilities.

Different observers have analysed haemoglobin. They find carbon, hydrogen, nitrogen, oxygen, sulphur, and iron. The percentage of

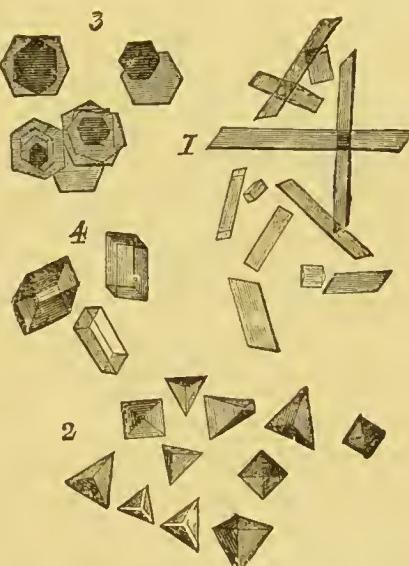


FIG. 30.—Oxyhaemoglobin crystals magnified: 1, from human blood; 2, from the guinea-pig; 3, squirrel; 4, hamster.

iron is 0·4. The amounts of the other elements are variously given, but, roughly, they are the same as in the proteids. We know at present as little of the chemical structure of haemoglobin as of the proteids generally.

On adding an acid or alkali to haemoglobin, it is broken up into two parts—a brown pigment called haeminatin, which contains all the iron of the original substance, and a proteid of the globulin class called globin. Haematin shows different spectroscopic appearances in acid and alkaline solutions, and yields certain products, which will be more fully studied in Lesson XX. For the present we will be content with investigating two of these, called haemin and haematoporphyrin.

**Haemin** is of great importance, as the obtaining of this substance in a crystalline form is the best chemical test for blood. Haemin crystals, sometimes called, after their discoverer, Teichmann's crystals,



FIG. 31.—Haemin crystals magnified. (Preyer.)

sodium chloride in addition. Fresh blood contains sufficient sodium chloride in itself.

The action of the acetic acid is (1) to split the haemoglobin into haeminatin and globin; and (2) to evolve hydrochloric acid from the sodium chloride. The haeminatin unites with the hydrochloric acid, and thus haemin is formed.

**Haematoporphyrin** is iron-free haematin; it may be prepared by mixing blood with strong sulphuric acid: the iron is taken out as ferrous sulphate. This substance is also found sometimes in nature; it occurs in certain invertebrate pigments, and may also be found in certain forms of pathological urine.

Haemoglobin may be estimated (1) by the amount of iron in the ash, or (2) by colorimetric methods, which are more fully described in the Appendix.

are from a chemical standpoint composed of the hydrochloride of haematin. They may be prepared for microscopic examination by boiling a fragment of dried blood with a drop of glacial acetic acid on a slide; on cooling, triclinic plates and prisms of a dark brown colour, often in star-shaped clusters and with rounded angles (fig. 31), separate out.

In the case of an old blood-stain it is necessary to add a crystal of

Hæmoglobin forms at least four compounds with gases :—

With oxygen	{ 1. Oxyhæmoglobin. { 2. Methæmoglobin.
With carbonic oxide.	3. Carbonic oxide hæmoglobin.
With nitric oxide.	4. Nitric oxide hæmoglobin.

These compounds have similar crystalline forms : they each probably consist of a molecule of hæmoglobin combined with one of the gases in question. They part with the combined gas somewhat readily ; but they are arranged in order of stability in the above list, the least stable first.

**Oxyhæmoglobin** is the compound that exists in arterial blood. Many of its properties have been already mentioned. The oxygen linked to the hæmoglobin, which is removed by the tissues through which the blood circulates, may be called the *respiratory oxygen of hæmoglobin*. The processes that occur in the lungs and tissues, resulting in the oxygenation and deoxygenation respectively of the hæmoglobin, may be imitated outside the body, using either blood or pure solutions of hæmoglobin. The respiratory oxygen can be removed, for example, in the Torricellian vacuum of a mercurial air-pump, or by passing a neutral gas like hydrogen through the blood, or by the use of reducing agents like ammonium sulphide or Stokes' reagent.<sup>1</sup> The older observers estimated that 1 gramme of hæmoglobin will combine with 1·6 c.c. of oxygen.<sup>2</sup>

If any of these methods for reducing oxyhæmoglobin is used, the bright red (arterial) colour of oxyhæmoglobin changes to the purplish (venous) tint of hæmoglobin. On once more allowing oxygen to come into contact with the hæmoglobin, as by shaking the solution with the air, the bright arterial colour returns.

These colour-changes may be more accurately studied with the spectroscope, and the constant position of the absorption bands seen constitutes an important test for blood pigment. It will be first necessary to describe briefly the instrument used.

**The Spectroscope.**—When a ray of white light is passed through a prism, it is refracted or bent at each surface of the prism ; the whole ray is, however, not equally bent, but it is split into its constituent colours, which may be allowed to fall on a screen. The band of colours beginning with the red, passing through orange, yellow, green,

<sup>1</sup> Stokes' reagent must always be freshly prepared : it is a solution of ferrous sulphate to which a little tartaric acid has been added, and then ammonia till the reaction is alkaline.

<sup>2</sup> Bohr has recently stated that oxyhæmoglobin is a mixture of several compounds of hæmoglobin with different amounts of oxygen in each.

blue, and ending with violet, is called a *spectrum*: this is seen in nature in the rainbow. It may be obtained artificially by the glass prism or prisms of a spectroscope.

The spectrum of sunlight is interrupted by numerous dark lines crossing it vertically called Frauenhofer's lines. These are perfectly constant in position, and serve as landmarks in the spectrum. The more prominent, lettered A, B, and C, are in the red; D, in the yellow; E, *b*, and F, in the green; G and H, in the violet. These lines are due to certain volatile substances in the solar atmosphere. If the light from burning sodium or its compounds be examined spectroscopically, it will be found to give a bright yellow line, or, rather, two bright yellow lines very close together. Potassium gives two bright red lines and one violet line; and the other elements, when incandescent, give characteristic lines, but none so

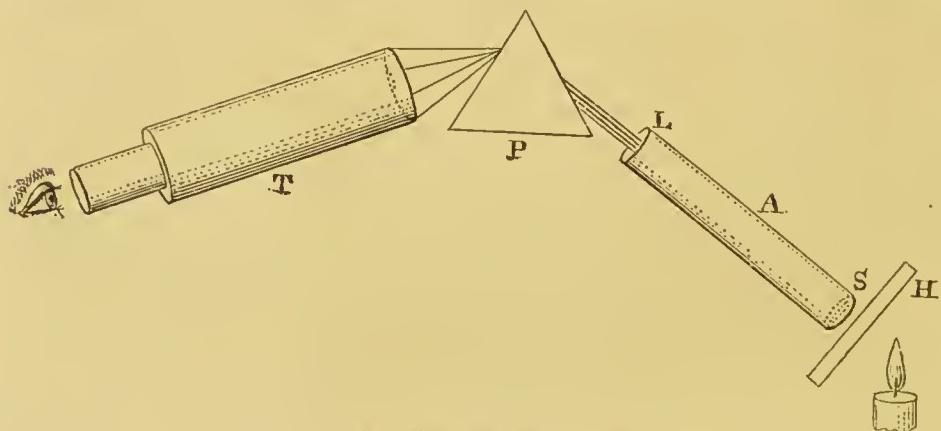


FIG. 32.—Diagram of spectroscope.

simple as sodium. If now the flame of a lamp be examined, it will be found to give a continuous spectrum like that of sunlight in the arrangement of its colours, but unlike it in the absence of dark lines; but if the light from the lamp be made to pass through sodium vapour before it reaches the spectroscope, the bright yellow light will be found absent, and in its place a dark line, or, rather, two dark lines very close together, occupying the same position as the two bright lines of the sodium spectrum. The sodium vapour thus absorbs the same rays as those which it itself produces at a higher temperature. Thus the D line, as we term it in the solar spectrum, is due to the presence of sodium vapour in the solar atmosphere. The other dark lines are similarly accounted for by other elements.

The large forms of spectroscope (fig. 32) consist of a tube A, called the collimator, with a slit at the end S, and a convex lens at the end L. The latter makes the rays of light passing through the slit from the

source of light parallel : they fall on the prism P, and then the spectrum so formed is focussed by the telescope T.

The third tube, D, seen in the next figure (fig. 33), carries a small transparent scale of wave-lengths, as in accurate observations the position of any point in the spectrum is given in the terms of the corresponding wave-lengths.

If we now interpose between the source of light and the slit S a piece of coloured glass (H in fig. 32), or a solution of a coloured substance contained in a vessel with parallel sides (the hæmatoscope of Herrmann, F in fig. 33), the spectrum is found to be no longer continuous, but is interrupted by a number of dark shadows, or

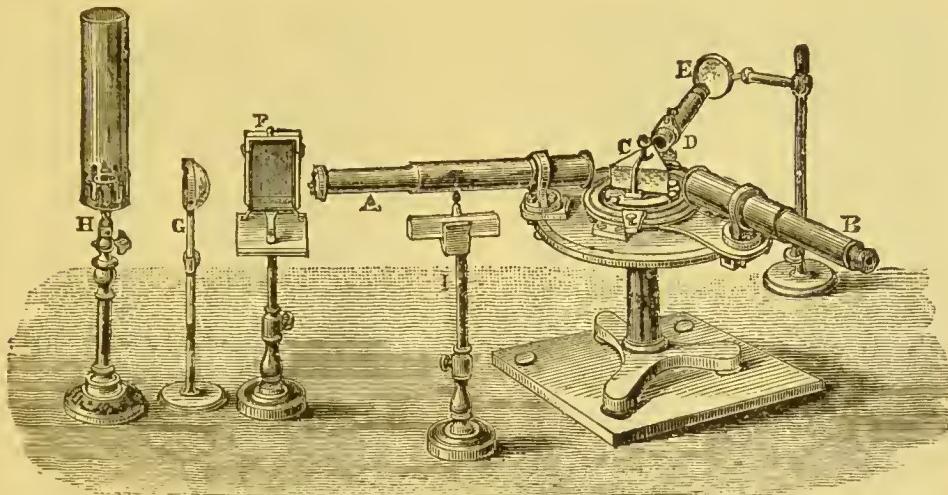


FIG. 33.—Spectroscope : A, collimator with adjustable slit at one (left) end and collimating lens at the other (right) end ; B, telescope moving on graduated arc divided into degrees ; C, prism or combination of prisms ; D, tube for scale ; E, mirror for illuminating scale ; F, vessel with parallel glass sides for holding fluid, shown with the flat side towards the reader ; G, long spectroscope bottle for examining a deep layer of fluid ; H, Argand burner ; I, long spectroscope bottle for examining a deep layer of fluid. (From a photograph taken by Dr. MacMunn, from McKendrie's "Physiology.")

*absorption bands* corresponding to the light absorbed by the coloured medium. Thus a solution of oxyhæmoglobin of a certain strength gives two bands between the D and E lines ; hæmoglobin gives only one ; and other red solutions, though to the naked eye similar to oxyhæmoglobin, will give characteristic bands in other positions.

A convenient form of small spectroscope is the *direct vision spectroscope*, in which, by an arrangement of alternating prisms of crown and flint glass, placed as in fig. 34, the spectrum is observed by the eye in the same line as the tube furnished with the slit—indeed slit and prisms are both contained in the same tube.

Such small spectrosopes may be used for class purposes, and may

for convenience be mounted on a stand provided with a gas-burner and a receptacle for the test-tube (see fig. 35).

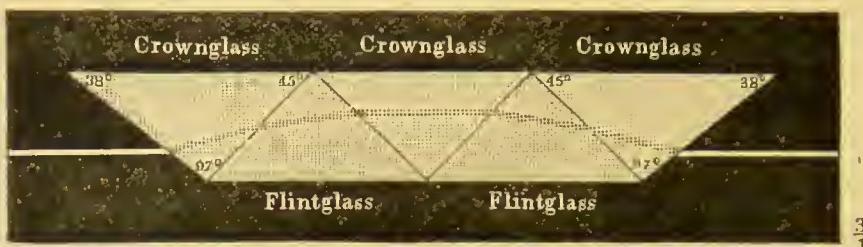


FIG. 34.—Arrangement of prisms in direct vision spectroscope.

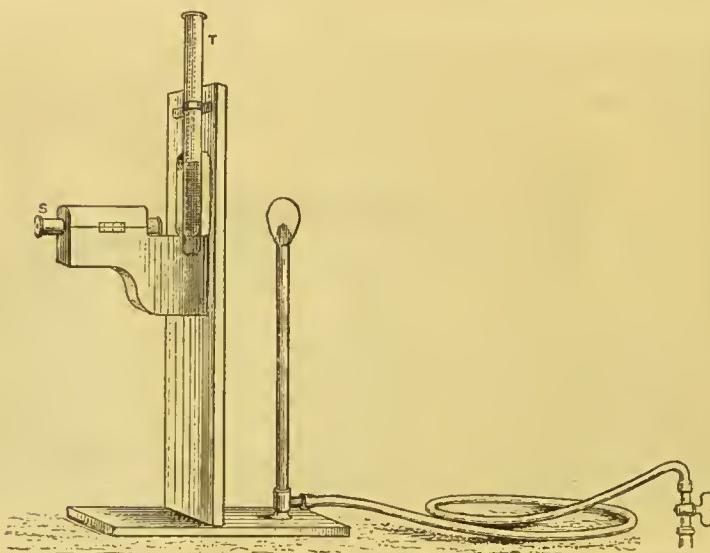


FIG. 35.—Stand for direct vision spectroscope : S, spectroscope ; T, test-tube for coloured substance under investigation.

The next figure illustrates a method of representing absorption spectra diagrammatically. The solution was examined in a layer 1 centimetre thick. The base line has on it at the proper distances the chief Fraunhofer lines, and along the right-hand edges are percentages of the amount of oxyhaemoglobin present in I, of haemoglobin in II. The width of the shadings at each level represents the position and amount of absorption corresponding to the percentages.

The characteristic spectrum of oxyhaemoglobin, as it actually appears through the spectroscope, is seen in the next figure (fig. 37, spectrum 2). There are two distinct absorption bands between the D and E lines; the one nearest to D (the  $\alpha$  band) being narrower, darker, and with better defined edges than the other (the  $\beta$  band). As will be seen on looking at fig. 36, a solution of oxyhaemoglobin of concentration greater than 0.65 per cent. and less than 0.85 per cent.

(examined in a cell of the usual thickness of 1 centimetre) gives one thick band overlapping both D and E, and a stronger solution only lets the red light through between C and D. A solution which gives the two characteristic bands must therefore be a dilute one. The one band ( $\gamma$  band) haemoglobin (fig. 37, spectrum 3) is not so well defined as the  $\alpha$  or  $\beta$  bands. On dilution it fades rapidly, so that in a solution of such strength that both bands of oxyhaemoglobin would be quite distinct the single band of haemoglobin has disappeared from view. The oxyhaemoglobin bands can be distinguished in a solution which contains only one part of the pigment to 10,000 of water, and even in

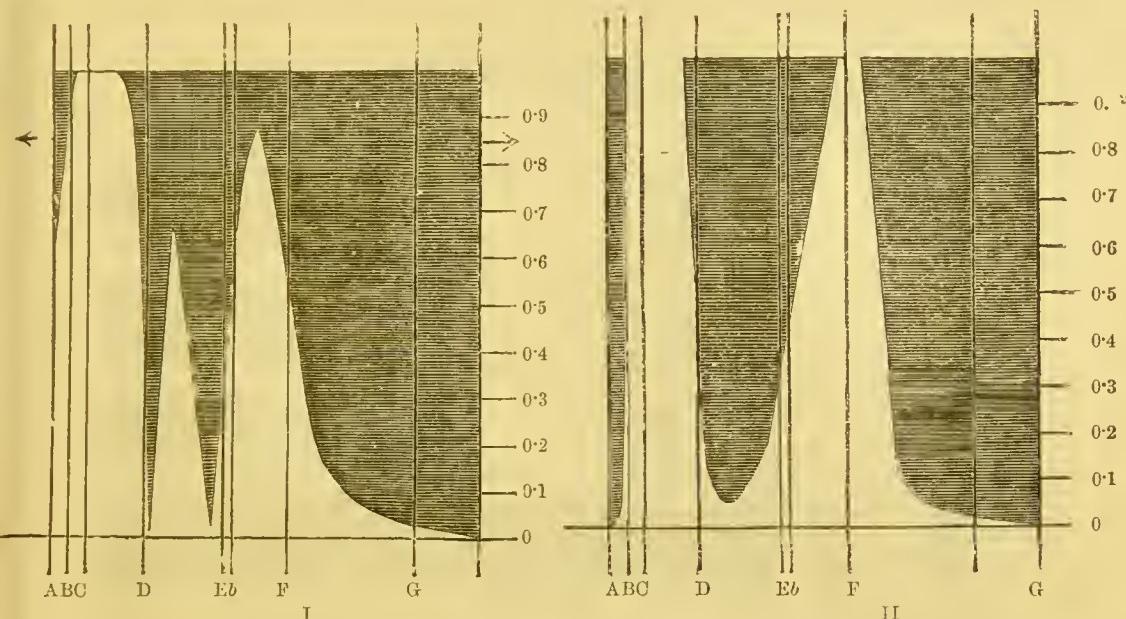


FIG. 36.—Graphic representations of the amount of absorption of light by solution of (I) oxyhaemoglobin, (II) of haemoglobin, of different strengths. The shading indicates the amount of absorption of the spectrum; the figures on the right border express percentages. (Rollett.)

more dilute solutions which seem to be colourless the  $\alpha$  band is still visible.

Compared to oxyhaemoglobin, the other compounds of haemoglobin with gases are comparatively unimportant. The main facts concerning each may be briefly stated as follows:—

**Methaemoglobin.**—This may be produced artificially in various ways, but as it also may occur in certain diseased conditions in the urine, it is of considerable practical importance. It can be crystallised, and is found to contain the same amount of oxygen as oxyhaemoglobin only combined more firmly. The oxygen is not removable by the air-pump, nor by a stream of a neutral gas like hydrogen. It can, however, by reducing agents like ammonium sulphide, be made to yield

haemoglobin. Methaemoglobin is of a mahogany-brown colour, and gives a characteristic absorption band in the red between the C and D lines (fig. 37, spectrum 5).

**Carbonic oxide haemoglobin** may be readily prepared by passing a stream of carbonic oxide through blood or through a solution of oxyhaemoglobin. It has a peculiar cherry-red colour. Its absorption spectrum is very like that of oxyhaemoglobin, but the two bands are slightly nearer the violet end of the spectrum (fig. 37, spectrum 4). Reducing agents, like ammonium sulphide, do not change it; the gas is more firmly combined than the oxygen in oxyhaemoglobin.

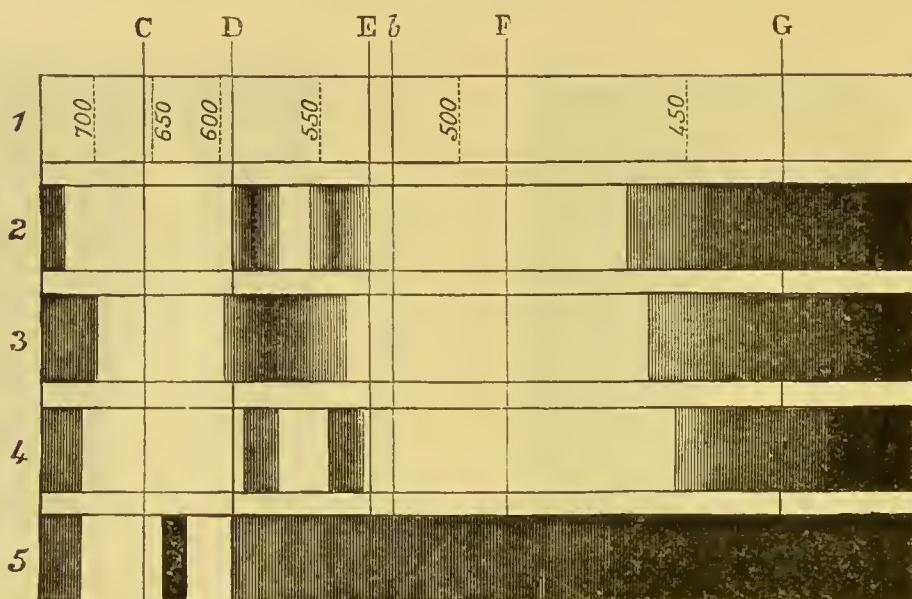


FIG. 37.—1, Solar spectrum ; 2, spectrum of oxyhaemoglobin (0·37 per cent. solution) : 3, spectrum of haemoglobin ; 4, spectrum of CO-haemoglobin ; 5, spectrum of methaemoglobin (concentrated solution).

CO-haemoglobin forms crystals like those of oxyhaemoglobin : it resists putrefaction for a very long time.

Carbonic oxide is given off during the imperfect combustion of carbon such as occurs in charcoal stoves : this acts as a powerful poison by combining with the haemoglobin of the blood, and thus interfering with normal respiratory processes. The colour of the blood and its resistance to reducing agents are in such cases characteristic.

**Nitric Oxide Haemoglobin.**—When ammonia is added to blood, and then a stream of nitric oxide passed through it, this compound is formed. It may be obtained in crystals isomorphous with oxy- and CO-haemoglobin. It also has a similar spectrum. It is even more stable than CO-haemoglobin ; it has no practical interest, but is only of theoretical importance as completing the series.

Recently C. Bøhr has advanced the theory that haemoglobin forms a compound with carbonic acid. He considers that the union is, like oxyhaemoglobin, a dissociable one, and that dissociation leading to evolution of the gas takes place in the blood vessels of the pulmonary alveoli. If this is really the case, haemoglobin appears to be not only an oxygen carrier but a carbonic-acid carrier. It has long been known that the red corpuscles contain carbonic acid, but it has always been supposed that this was not in actual combination with the pigment; and even Bohr does not consider that the gas is united to the haemoglobin in the same way as oxygen is. Perhaps it may be united to the proteid globin rather than to the iron-containing constituent. The subject, however, cannot yet be considered settled.  $\text{CO}_2$ -haemoglobin, if it does exist, shows no spectroscopic differences from haemoglobin; and no one disputes that there is, in addition to the carbonic acid of the corpuscles, a much larger amount dissolved in the plasma, chiefly in the form of carbonates and bicarbonates.

### CHEMISTRY OF RESPIRATION

The consideration of the blood, and especially of its pigment, is so closely associated with respiration that a brief account of that process follows conveniently here.

The lungs consist essentially of numerous little hollow sacs, in the walls of which is a close plexus of capillary blood vessels. These air cells, or alveoli, communicate with the external air by the trachea, bronchi, and bronchial tubes. Inspiration is due to a muscular effort that enlarges the thorax, the closed cavity in which the lungs are situated. Owing to the atmospheric pressure the lungs become distended. The atmospheric air does not, however, actually penetrate beyond the largest bronchial tubes; the gases which get into the smaller tubes and air cells do so by diffusion. Expiration is ordinarily brought about by the elastic rebound of the lungs and chest walls, and is only a muscular effort when forced; but even the most vigorous expiratory effort is unable to expel the alveolar air. This air and the blood in the capillaries are only separated by the thin capillary and alveolar walls. The blood parts with its excess of carbonic acid and watery vapour to the alveolar air; the blood at the same time receives from the alveolar air a supply of oxygen which renders it arterial.

The intake of oxygen is the commencement, and the output of carbonic acid the end, of the series of changes known as respiration. The intermediate steps take place all over the body, and constitute what is known as *tissue respiration*. We have already seen that oxyhaemoglobin is only a loose compound, and in the tissues it parts with its oxygen. The oxygen does not necessarily undergo immediate union with carbon to form carbonic acid, and with hydrogen to form water,

but in most cases, as in muscle, is held in reserve by the tissue itself. Ultimately, however, these two oxides are formed : they are the chief products of combustion. Certain other products which represent the combustion of nitrogenous material (urea, uric acid, &c.) ultimately leave the body in the urine. All these substances pass into the venous blood, and the gaseous products, carbonic acid, and a portion of the water find an outlet by the lungs.

**Inspired and Expired Air.**—The composition of the inspired or atmospheric air and the expired air may be compared in the following table :—

		Inspired Air	Expired Air
Oxygen . . . .		20.96 vols. per cent.	16.6 vols. per cent.
Nitrogen . . . .		79 "	79 "
Carbonic acid . . . .		0.04 "	4.4 "
Watery vapour . . . .		variable	saturated
Temperature . . . .		"	that of body (36° C.)

The nitrogen remains unchanged. The chief change is in the proportion of oxygen and carbonic acid. The loss of oxygen is about 5, the gain in carbonic acid  $4\frac{1}{2}$ . If the inspired and expired airs are carefully measured at the same temperature and barometric pressure, the volume of expired air is thus rather less than that of the inspired. The conversion of oxygen into carbonic acid would not cause any change in the volume of the gas, for a molecule of oxygen ( $O_2$ ) would give rise to a molecule of carbonic acid ( $CO_2$ ), which would occupy the same volume. It must, however, be remembered that carbon is not the only element which is oxidised. Fats contain a number of atoms of hydrogen which during metabolism are oxidised to form water ; a certain small amount of oxygen is also used in the formation of urea. Carbohydrates contain sufficient oxygen in their own molecules to oxidise their hydrogen ; hence the apparent loss of oxygen is least when a vegetable diet (that is, one consisting largely of starch and other carbohydrates) is taken, and greatest when much fat and proteid are eaten. The quotient  $\frac{CO_2 \text{ given off}}{O_2 \text{ absorbed}}$  is called the *respiratory quotient*. Normally it is  $\frac{4.5}{5} = 0.9$ , but this varies considerably with diet, as just stated. It varies also with muscular exercise, when the output of carbonic acid is much increased.

**Gases of the Blood.**—From 100 volumes of blood about 60 volumes of gas can be removed by the mercurial air-pump. The average composition of this gas in dog's blood is—

		Arterial Blood	Venous Blood
Oxygen . . . .		20	8 to 12
Nitrogen . . . .		1 to 2	1 to 2
Carbonic acid . . .		40	46

The nitrogen in the blood is simply dissolved from the air just as water would dissolve it: it has no physiological importance. The other two gases are present in much greater amount than can be explained by simple solution; they are, in fact, chiefly present in loose chemical compounds. Less than one volume of the oxygen and about two of carbonic acid are present in simple solution in the plasma.

**Oxygen in the Blood.**—The amount of gas dissolved in a liquid varies with the pressure of the gas; double the pressure and the amount of gas dissolved is doubled. Now this does not occur in the case of oxygen and blood; very nearly the same amount of oxygen is dissolved whatever be the pressure. We thus have a proof that oxygen is not merely dissolved in the blood, but is in chemical union; and the fact that the oxygen of oxyhaemoglobin can be replaced by equivalent quantities of other gases, like carbonic oxide, is a further proof of the same statement. The tension or partial pressure of oxygen in the air of the alveoli is less than that in the atmosphere, but greater than that in venous blood; hence oxygen passes from the alveolar air into the blood; the oxygen immediately combines with the haemoglobin, and thus leaves the plasma free to absorb more oxygen; and this goes on until the haemoglobin is entirely, or almost entirely, saturated with oxygen. The reverse change occurs in the tissues when the partial pressure of oxygen is lower than in the plasma, or in the lymph that bathes the tissue elements; the plasma parts with its oxygen to the lymph, the lymph to the tissues; the oxyhaemoglobin then undergoes dissociation to supply more oxygen to the plasma and lymph, and this in turn to the tissues once more. This goes on until the oxyhaemoglobin loses a great portion of its store of oxygen, but even in asphyxia it does not lose all.

The avidity of the tissues for oxygen is shown by Ehrlich's experiments with methylene blue and similar pigments. Methylene blue is more stable than oxyhaemoglobin; but if it is injected into the circulation of a living animal, and the animal killed a few minutes later, the blood is found dark blue, but the organs colourless. On exposure to oxygen the organs become blue. In other words, the tissues have removed the oxygen from methylene blue to form a colourless reduction product; on exposure to the air this once more unites with oxygen to form methylene blue.

**Carbonic Acid in the Blood.**—What has been said for oxygen holds good in the reverse direction for carbonic acid. Compounds are formed in the tissues where the tension of the gas is high: these pass into the lymph, then into the blood, and in the lungs the compounds undergo dissociation, carbonic acid passing into the air of the alveolar air, where the tension of the gas is comparatively low, though it is greater here than in the expired air.

The relations of this gas and the compounds it forms are more complex than in the case of oxygen. If blood is divided into plasma and corpuscles, it will be found that both yield carbonic acid, but the yield from the plasma is the greater. If we place blood in a vacuum it bubbles, and gives out all its gases; addition of a weak acid causes no further liberation of carbonic acid. But when plasma or serum is similarly treated the gas comes off, but about 5 per cent. of the carbonic acid is fixed—that is, requires the addition of some stronger acid, like phosphoric acid, to displace it. Fresh red corpuscles will, however, take the place of the phosphoric acid, and thus it has been surmised that oxyhaemoglobin has the properties of an acid.

One hundred volumes of venous blood contain forty-six volumes of carbonic acid. Whether this is in solution or in chemical combination is determined by ascertaining the tension of the gas in the blood. One hundred volumes of blood plasma would dissolve fifty volumes of the gas at atmospheric pressure, if its solubility in plasma were equal to that in water. If, then, the carbonic acid were in a state of solution, its tension would be equal to that of the atmosphere, but it proved to be only equal to 5 per cent. of an atmosphere. This means that when venous blood is brought into an atmosphere containing 5 per cent. of carbonic acid, the blood neither gives off any carbonic acid nor takes up any from the atmosphere. Hence the remainder of the gas, 95 per cent., is in a condition of chemical combination. The chief compound appears to be sodium bicarbonate.

Bohr's recent researches have shown that probably the purely physical process of diffusion will not satisfactorily explain the manner in which carbonic acid is discharged from the blood into the alveolar air. Much recent physiological research has shown that we must largely abandon physical theories for what are called vitalistic theories; in other words, the vital processes of selection possessed by living protoplasm may counteract or supplement physical processes. Thus, in our study of absorption, we have seen that mere filtration or diffusion will not explain the passage of the digested foods into the blood stream or the lacteals. Heidenhain has further shown that lymph is not merely a transudation from blood plasma through the

thin walls of the capillary blood vessels. Sugar injected into the blood passes into the lymph, but soon the percentage in the lymph is found to be higher than in the blood—a phenomenon which can only be ascribed to the secretory activity of the endothelial cells of the vessel walls. In the same way it seems that the discharge of carbonic acid from the blood is dependent on the secretory activity of the epithelium lining the pulmonary alveoli. If Bohr's figures are ultimately found to be correct, it is difficult to avoid such a conclusion. His figures from the dog are :—

Tension of carbonic acid in alveolar air . . . .	3·56 per cent.
" " " venous blood : : :	5·4 "
" " " arterial " . . . :	2·8 "

If we compare the tensions of the gas in venous blood and alveolar air, we see that in accordance with the laws of diffusion the carbonic acid has passed from a region of high to one of low tension. But, on looking at the figure for arterial blood, this change has gone on beyond the establishment of equilibrium, and that, in fact, the carbonic acid has passed from a medium where its tension is low to one where its tension is higher.

Certain Continental observers have stated that certain noxious substances are ordinarily contained in expired air which are much more poisonous than carbonic acid, but researches in this country have entirely failed to substantiate this. If precautions be taken by absolute cleanliness to prevent admixture of the air with exhalations from skin and clothes, the expired air only contains one noxious substance, and that is carbonic acid.

**Tissue-Respiration.**—Before the processes of respiration were fully understood the lungs were looked upon as the seat of combustion; they were regarded as the stove for the rest of the body where effete material was brought by the venous blood to be burnt up. The impossibility of this was shown when it was pointed out that, if all the heat of the body was produced in the lungs, their temperature would be raised so high as to destroy them.

Physiologists next transferred the seat of the combustion to the blood; but since then innumerable facts and experiments have shown that it is the tissues themselves, and not the blood, where combustion occurs. The methylene-blue experiments already described (p. 83) show this; and the following experiment is also quite conclusive. A frog can be kept alive for some time after salt solution is substituted for its blood. The metabolism goes on actively if the animal is kept in pure oxygen. The taking up of oxygen and giving out of carbonic acid must therefore occur in the tissues, as the animal has no blood.

## LESSON X

## URINE

1. Test the reaction of urine to litmus paper.
2. Determine its specific gravity by the urinometer.
3. Test for the following **inorganic salts** :—

(a) *Chlorides*.—Acidulate with nitric acid and add silver nitrate ; a white precipitate of silver chloride, soluble in ammonia, is produced. The object of acidulating with nitric acid is to prevent phosphates being precipitated by the nitrate of silver.

(b) *Sulphates*.—Acidulate with hydrochloric acid and add barium chloride. A white precipitate of barium sulphate is produced. Hydrochloric acid is again added first to prevent precipitation of phosphates.

(c) *Phosphates*.—i. Add ammonia ; on standing a white flocculent precipitate of earthy (that is, calcium and magnesium) phosphates is produced. The alkaline (that is, sodium and potassium) phosphates remain in solution.

ii. Mix another portion of urine with half its volume of nitric acid ; add ammonium molybdate, and boil. A yellow crystalline precipitate falls.

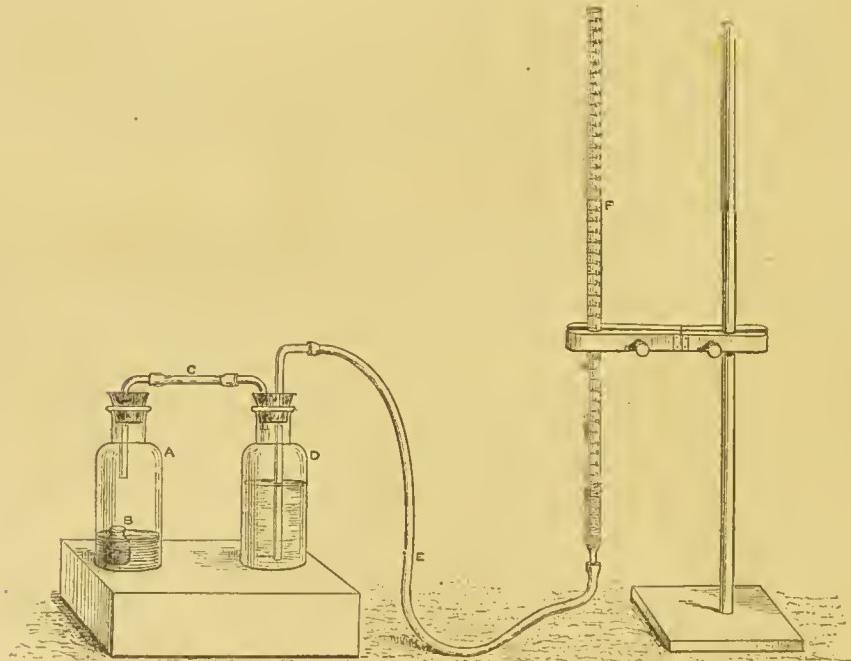


FIG. 38.—Simple urea apparatus.

4. *Urea*.—Take some urea crystals. Observe that they are readily soluble in water, and that effervescence occurs when fuming nitric acid is added to the solution. The effervescence is due to the breaking up of the urea.

Carbonic acid and nitrogen come off. A similar bubbling, due to evolution of nitrogen, occurs when an alkaline solution of sodium hypobromite is added to another portion of the solution.

5. Heat some urea crystals in a test-tube. Biuret is formed, and ammonia comes off. Add a drop of copper-sulphate solution and a few drops of potash. A rose-red colour is produced.

#### 6. Quantitative estimation of urea.

The burette F is connected by the tube E to the bottle D, as in the figure, both D and F being filled with water to the same level. The bottle A contains 25 c.e. of alkaline solution of sodium-hypobromite (made by mixing 2 e.e. of bromine to 23 c.c. of a 40-per-cent. solution of caustic soda). Measure 5 c.c. of urine into the little bottle B, and carefully lower it into A. Cork A tightly, and connect it by the tube C to the bottle D, as in the figure. Bring the water in the bottle D and the burette F to the same level, and then read the burette. By inclining A, mix the urine and hypobromite together. Cool A by immersing it in a vessel of water. The carbonic acid resulting from the decomposition of urea is absorbed by the excess of soda in A; the nitrogen is evolved and pushes the water in D into the burette. Carefully measure the water so displaced, bringing the water in D and F to the same level. Its volume is equal to that of the nitrogen evolved: 35·4 c.c. of nitrogen are yielded by 0·1 gramme of urea. From this the quantity of urea in the 5 c.e. of urine and the percentage of urea can be calculated. If the total urea passed in the twenty-four hours is to be ascertained, the twenty-four hours' urine must be carefully measured and thoroughly mixed. A sample is then taken from the total for analysis; and then, by a simple sum in proportion, the total amount of urea is ascertained.

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The kidney is a compound tubular gland, the tubules of which it is composed differing much in the character of the epithelium that lines them in various parts of their course. The true secreting part of the kidney is the glandular epithelium that lines the convoluted portions of the tubules; there is in addition to this what is usually termed the filtering apparatus: tufts of capillary blood vessels called the Malpighian glomeruli are supplied with afferent vessels from the renal artery; the efferent vessels that leave these have a smaller calibre, and thus there is high pressure in the Malpighian capillaries. Certain constituents of the blood, especially water and salts, pass through the thin walls of these vessels into the surrounding Bowman's capsule which forms the commencement of each renal tubule. Bowman's capsule is lined by a flattened epithelium, which is reflected over the capillary tuft. Though the process which occurs here is generally spoken of as a filtration, yet it is no purely mechanical process, but the cells exercise a selective influence, and prevent the albuminous constituents of the blood from escaping. During the passage of the water which leaves the blood at the glomerulus through the rest of the renal tubule, it is in part reabsorbed; the urine also gains the constituents urea, urates, &c., which are poured into it by the secreting cells of the convoluted tubules.

The term *excretion* is better than *secretion* as applied to the kidney, for the constituents of the urine are not actually formed in the kidney itself (as, for instance, the bile is formed in the liver), but they are formed elsewhere ; the kidney is simply the place where they are picked out from the blood and eliminated from the body.

### GENERAL CHARACTERS OF URINE

**Quantity.**—A man of average weight and height passes from 1400 to 1600 c.c., or about 50 oz. daily. This contains about 50 grammes ( $1\frac{1}{2}$  oz.) of solids. The urine should be collected in a tall glass vessel capable of holding 3000 c.c., which should have a smooth-edged neck accurately covered by a ground glass plate to exclude dust and avoid evaporation. The vessel, moreover, should be graduated so that the amount may be easily read off. From the total quantity thus collected in the twenty-four hours samples should be drawn off for examination.

**Colour.**—This is some shade of yellow which varies considerably in health with the concentration of the urine. It appears to be due to a mixture of pigments, of which urobilin is the only one of which we have any accurate knowledge. Urobilin is undoubtedly derived from the blood pigment, and, like bile pigment, is an iron-free derivative of haemoglobin. The theory usually accepted concerning its mode of origin is that bile pigment is in the intestines converted into stercobilin ; that most of the stercobilin leaves the body with the faeces ; that some is reabsorbed and is excreted with the urine as urobilin. Both stercobilin and urobilin are very like the artificial reduction product of bilirubin called hydrobilirubin (see p. 60).

**Reaction.**—The reaction of normal urine is acid. This is not due to free acid, as the uric and hippuric acids in the urine are combined as urates and hippurates respectively. The acidity is due to acid sodium phosphate. Under certain circumstances the urine becomes less acid and even alkaline ; the most important of these are as follows :—

1. During digestion. Here there is a formation of free acid in the stomach, and a corresponding liberation of bases in the blood which passing into the urine diminish its acidity, or even render it alkaline. This is called *the alkaline tide* ; the opposite condition, *the acid tide*, occurs after a fast—for instance, before breakfast.

2. In herbivorous animals and vegetarians. The food here contains excess of alkaline salts of acids like tartaric, citric, malic, &c. These acids are oxidised into carbonates, which passing into the urine give it an alkaline reaction.

**Specific Gravity.**—This should be taken in a sample of the twenty-four hours' urine with a good urinometer (see fig. 39).

The specific gravity varies inversely as the quantity of urine passed under normal conditions from 1015 to 1025. A specific gravity below 1010 should excite suspicion of hydruria ; one over 1030, of a febrile condition, or diabetes, a disease in which it may rise to 1050. The specific gravity has, however, been known to sink as low as

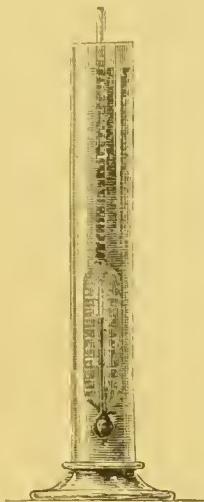


FIG. 39.—Urinometer floating in urine in a testing glass.

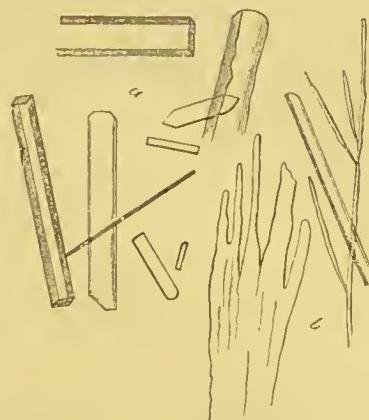


FIG. 40.—Crystals of urea : *a*, four-sided prisms ; *b*, indefinite crystals, such as are usually formed from alcohol solutions.

1002 (after large potations, *urina potus*), or to rise as high as 1035 (after great sweating) in perfectly healthy persons.

**Composition.**—The following table gives the average amounts of the urinary constituents passed by a man in the twenty-four hours :—

Water	.	.	.	.	1500·00	grammes
Total solids	:	.	.	.	72·00	"
Urea	.	.	.	.	33·18	"
Uric acid	.	.	.	.	0·55	"
Hippuric acid	.	.	.	.	0·40	"
Creatinine	.	.	.	.	0·91	"
Pigment and other organic substances	.	.	.	.	10·00	"
Sulphuric acid	.	.	.	.	2·01	"
Phosphoric acid	.	.	.	.	3·16	"
Chlorine	.	.	.	.	7·50	"
Ammonia	.	.	.	.	0·77	"
Potassium	.	.	.	.	2·50	"
Sodium	.	.	.	.	11·09	"
Calcium	.	.	.	.	0·26	"
Magnesium	.	.	.	.	0·21	"

The most abundant constituents of the urine are water, urea, and sodium chloride. In the foregoing table the student must not be misled by seeing the names of the acids and metals separated. The acids and the bases are combined to form salts, urates, chlorides, sulphates, phosphates, &c.

## UREA

**Urea, or Carbamide,  $\text{CO}(\text{NH}_2)_2$ , is isomeric (that is, has the same empirical, but not the same structural formula) with ammonium cyanate ( $\text{NH}_4\text{CNO}$ ), from which it was first prepared synthetically by Wöhler in 1828. Since then it has been prepared synthetically in other ways. Wöhler's observation derives interest from the fact that this was the first organic substance which was prepared synthetically by chemists.**

It may be crystallised out from the urine by a rather complicated process (see Lesson XXVI.), and it is then found to be readily soluble

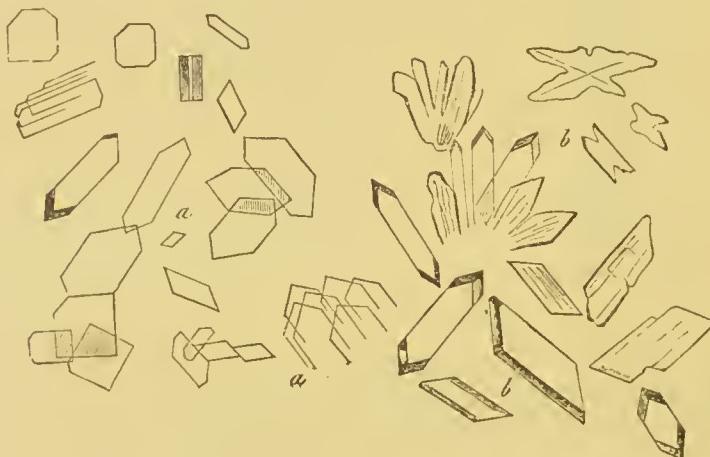


FIG. 41.—*a*, nitrate; *b*, oxalate of urea.

both in water and alcohol: it has a saltish taste, and is neutral to litmus paper. The form of its crystals is shown in fig. 40.

When treated with nitric acid, nitrate of urea ( $\text{CON}_2\text{H}_4\cdot\text{HNO}_3$ ) is formed; this crystallises in octahedra, lozenge-shaped tablets, or hexagons (fig. 41, *a*). When treated with oxalic acid, flat or prismatic crystals of urea oxalate ( $\text{CON}_2\text{H}_4\cdot\text{H}_2\text{C}_2\text{O}_4 + \text{H}_2\text{O}$ ) are formed (fig. 41, *b*).

These crystals may be readily obtained in an impure form by adding large excess of the respective acids to urine which has been concentrated to a third or a quarter of its bulk.<sup>1</sup>

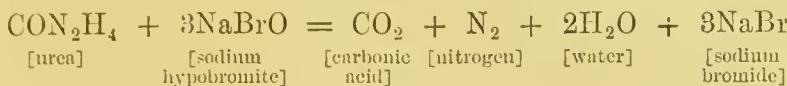
Under the influence of an organised ferment, the torula or micrococcus ureæ, which grows readily in stale urine, urea takes up water, and is converted into ammonium carbonate [ $\text{CON}_2\text{H}_4 + 2\text{H}_2\text{O} = (\text{NH}_4)_2\text{CO}_3$ ]. Hence the ammoniacal odour of putrid urine.

By means of nitrous acid, urea is broken up into carbonic acid,

<sup>1</sup> The preparation of urea nitrate is postponed to the next lesson, when other microscopic crystals will also be under examination.

water, and nitrogen,  $\text{CON}_2\text{H}_4 + 2\text{HNO}_2 = \text{CO}_2 + 3\text{H}_2\text{O} + 2\text{N}_2$ . This may be used as a test for urea. Add fuming nitric acid to a solution of urea, or to urine; an abundant evolution of gas bubbles takes place.

Hypobromite of soda decomposes urea in the following way :—



This reaction is important, for on it one of the best methods for estimating urea depends. There have been various pieces of apparatus invented for rendering the analysis easy; but the one described in the practical exercise at the head of this lesson can be easily made from the bottles and burettes which are always at hand in a laboratory. The stoppers to the bottles must not be leaky, or some of the nitrogen will be lost. If the experiment is performed as directed, nitrogen is the only gas that comes off, the carbonic acid being absorbed by excess of soda. The amount of nitrogen is a measure of the amount of urea.

The quantity of urea is somewhat variable, the chief cause of variation being the amount of proteid food ingested. In a man in a state of equilibrium the quantity of urea secreted daily averages 33 grammes (500 grains). The normal percentage in human urine is 2 per cent.; but this also varies, because the concentration of the urine varies considerably in health. In dogs it may be 10 per cent. The excretion of urea is usually at a maximum three hours after a meal, especially after a meal rich in proteids. The urea does not come, however, direct from the food; the food must be first assimilated, and become part of the body before it can break down to form urea. An exception to this rule is to be found in the case of the amido-acids, especially leucine, which are formed in the intestinal canal from proteids during digestion. Leucine is carried to the liver, and is converted into urea; but only a very small fraction of the urea in the urine is formed in this way. Urea is increased by food first because food stimulates the tissues to activity, and so metabolism is increased, and secondly it stimulates the kidney to activity, and so waste accumulated products are got rid of. The more proteid food is given, the more waste products do the tissues discharge from their protoplasm, in order to make room for the new proteid which is built into their protoplasm, to be later on displaced in the same way.

Muscular exercise has little effect on the amount of urea discharged. In very intense muscular work there is a slight increase of urea, but this is quite insignificant when compared to the increase of work. This is strikingly different from what occurs in the case of

carbonic acid ; the more the muscles work, the more carbonic acid do they send into the venous blood, and the more carbonic acid finds its way into the expired air. Muscular work falls upon the carbon rather than on the nitrogen of the muscle substance.

**Where is Urea formed?**—The older authors considered that it was formed in the kidneys, just as they also erroneously thought that carbonic acid was formed in the lungs. Prévost and Dumas were the first to show that after complete extirpation of the kidneys the formation of urea goes on, and that it accumulates in the blood and tissues. Similarly, in those cases of disease in which the kidneys cease work, urea is still formed and accumulates. This condition is called uræmia (or urea in the blood), and unless the urea be discharged from the body the patient dies. There is no doubt, however, that it is not urea but some antecedent of urea that acts most poisonously, and is the cause of death.

If, then, the kidneys are not specially the seat of urea formation, where is this special seat, or is there any special seat ? There can be little doubt that urea is formed in all tissues of the body in which proteids are present. It may not be that the formation of urea is perfected in each tissue, for if we look to the most abundant tissue, the muscular tissue, urea is absent, or nearly so. Yet there can be no doubt that the chief place from which urea ultimately comes is the muscular tissue. Some intermediate step occurs in the muscles ; the final steps occur elsewhere.

In muscles we find a substance called creatine in fairly large quantities. If creatine is injected into the blood it is discharged as creatinine. But there is very little creatinine in normal urine ; what little there is is accounted for by the creatine in the food ; the muscular creatine is discharged as urea ; in fact, urea can be artificially obtained from creatine in the laboratory.

Similarly, other cellular organs, spleen, lymphatic glands, secreting glands, participate in the formation of urea ; but the most important appears to be the liver : this is the organ where the final changes take place. The urea is then carried by the blood to the kidney, and is there excreted.

The facts of pathology point very strongly in support of the theory that urea is formed in the liver. The principal are the following :—

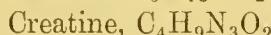
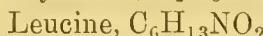
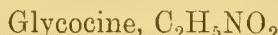
1. In diabetes the metabolic processes in the liver are much increased ; there is sugar in the urine, and also a larger amount of urea than normal.

2. In opposite conditions, when degenerative processes occur in the liver, there is a lessened formation of urea. This is very marked in

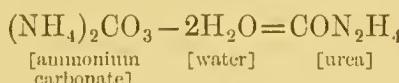
a disease called *acute yellow atrophy*; here leucine and tyrosine are found in the urine, and urea is almost absent. In cirrhosis of the liver the urea is also much lessened, and its place is taken by ammonia.

The next question that confronts us is, What are the intermediate stages between proteid and urea? This question is difficult to answer. Ordinary chemical processes carried out in the laboratory do not lead to the formation of urea from proteid; and it is only quite recently that Drechsel has by new methods succeeded in artificially producing urea from casein. We have already seen from theoretical considerations that creatine is probably one of the principal intermediate stages; uric acid is perhaps another. At any rate, chemists can obtain urea artificially from both creatine and uric acid. Other amido-acids, such as glycocine (amido-acetic acid) and leucine (amido-caproic acid), are probably to be included in the same category. The introduction of glycocine or leucine into the bowel, or into the circulation, leads to an increase of urea in the urine; and doubtless a small quantity of urea normally originates from the leucine formed during pancreatic digestion. There is no evidence that tyrosine acts in the same way. In acute yellow atrophy the appearance of amido-acids in the urine in place of urea lends support to this theory.

If, now, we glance at the formulae of these amido-acids—



—we see that the carbon atoms are more numerous than the nitrogen atoms. In urea,  $\text{CON}_2\text{H}_4$ , the reverse is the case. The amido-acids must therefore be split into simpler compounds, which unite with one another to form urea. Urea formation is thus, in part, synthetic. There have been various theories advanced as to what these simpler compounds are. Some have considered that cyanate, others that carbamate, and others still that carbonate of ammonium is formed. Schröder's recent work proves that ammonium carbonate is one of the urea precursors, if not the principal one. The equation which represents the reaction is as follows:—



Schröder's principal experiment was this: a mixture of blood and ammonium carbonate was injected into the liver by the portal vein; the blood leaving the liver by the hepatic vein was found to contain

urea in great abundance. This does not occur when the same experiment is performed with any other organ of the body, so that Schröder's experiments also prove the great importance of the liver in urea formation.

### THE INORGANIC CONSTITUENTS OF URINE

The inorganic or mineral constituents of urine are chiefly chlorides, phosphates, sulphates, and carbonates ; the metals with which these are in combination are sodium, potassium, ammonium, calcium, and magnesium. The total amount of these salts varies from 9 to 25 grammes daily. The most abundant is sodium chloride, which averages in amount 10 to 13 grammes per diem. These substances are derived from two sources—first from the food, and secondly as the result of metabolic processes. The chlorides and most of the phosphates come from the food ; the sulphates and some of the phosphates, as a result of metabolism. The salts of the blood and of the urine are much the same, with the important exception that, whereas the blood contains only traces of sulphates, the urine contains abundance of these salts. The sulphates are derived from the changes that occur in the proteids of the body ; the nitrogen of proteids leaves the body as urea and uric acid ; the sulphur of the proteids is oxidised to form sulphuric acid, which passes into the urine in the form of sulphates. The excretion of sulphates, moreover, runs parallel to that of urea. The chief tests for the various salts have been given in the practical exercises at the head of this lesson.

**Chlorides.**—The chief chloride is that of sodium. The ingestion of sodium chloride is followed by its appearance in the urine, some on the same day, some on the next day. Some is decomposed to form the hydrochloric acid of the gastric juice. The salt, in passing through the body, fulfils the useful office of stimulating metabolism and secretion.

**Sulphates.**—The sulphates in the urine are principally those of potassium and sodium. They are derived from the metabolism of proteids in the body. Only the smallest trace enters the body with the food. Sulphates have an unpleasant bitter taste (for instance, Epsom salts) ; hence we do not take food that contains them. The sulphates vary in amount from 1·5 to 3 grammes daily.

In addition to these sulphates there is a small quantity, about one-tenth of the total sulphates, that are combined with organic radicles ; these are known as ethereal sulphates, and they originate from putrefactive processes occurring in the intestine. The chief of these

ethereal sulphates are phenol sulphate of potassium and indoxylo sulphate of potassium. The latter originates from the indole formed in the intestine, and as it yields indigo when treated with certain reagents it is sometimes called *indican*. It is very important to remember that the indican of urine is not the same thing as the indican of plants. Both yield indigo, but there the resemblance ceases.

**Carbonates.**—Carbonates and bicarbonates of sodium, calcium, magnesium, and ammonium are generally present in alkaline urine. They arise from the carbonates of the food, or from vegetable acids (malic, tartaric, &c.) in the food. They are, therefore, more abundant in the urine of herbivora and vegetarians, whose urine is thus rendered alkaline. Urine containing carbonates becomes, like saliva, cloudy on standing, the precipitate consisting of calcium carbonate, and also phosphates.

**Phosphates.**—Two classes of phosphates occur in normal urine :—

(1) Alkaline phosphates—that is, phosphates of sodium (abundant) and potassium (scanty).

(2) Earthy phosphates—that is, phosphates of calcium (abundant) and magnesium (scanty).

The composition of the phosphates in urine is liable to variation. In acid urine the acid salts are generally present, and give the urine an acid reaction. These are chiefly—

Sodium dihydrogen phosphate,  $\text{NaH}_2\text{PO}_4$ , and calcium dihydrogen phosphate,  $\text{Ca}(\text{H}_2\text{PO}_4)_2$ .

In neutral urine, in addition, disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4$ ), calcium hydrogen phosphate,  $\text{CaHPO}_4$ , and magnesium hydrogen phosphate,  $\text{MgHPO}_4$ , are found. In alkaline urine there may be instead of, or addition to, the above the normal phosphates of sodium, calcium, and magnesium [ $\text{Na}_3\text{PO}_4$ ,  $\text{Ca}_3(\text{PO}_4)_2$ ,  $\text{Mg}_3(\text{PO}_4)_2$ ].

The earthy phosphates are precipitated by rendering the urine alkaline by ammonia. In decomposing urine ammonia is formed from the urea : this also precipitates the earthy phosphates. The phosphates most frequently found in the white creamy precipitate which occurs in decomposing urine are—

(1) Triple phosphate or ammonio-magnesium phosphate ( $\text{NH}_4\text{MgPO}_4 + 6\text{H}_2\text{O}$ ). This crystallises in ‘coffin-lid’ crystals (see fig. 42) or feathery stars.

(2) Stellar phosphate, or calcium phosphate, which crystallises in star-like clusters of prisms.

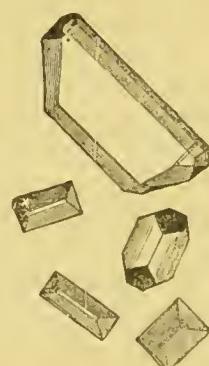


FIG. 42.—Ammonio-magnesium or triple phosphate.

Normal urine gives no precipitate when it is boiled. Neutral, alkaline, and occasionally faintly acid urines give a precipitate of calcium phosphate when boiled : this precipitate is amorphous, and is liable to be mistaken for albumin. It may be distinguished readily from albumin, as it is soluble in a few drops of acetic acid, whereas coagulated proteid does not dissolve.

The phosphoric acid in the urine chiefly originates from the food, but is partly a decomposition product of the phosphorised organic materials in the body, such as lecithin and nuclein. The amount of  $P_2O_5$  in the twenty-four hours' urine varies from 2·5 to 3·5 grammes, of which the earthy phosphates contain about half (1 to 1·5 gr.).

## LESSON XI

URINE (*continued*)

1. **Urea Nitrate.**—Evaporate some urine to a quarter of its bulk. Pour the concentrated urine into a watch-glass; let it cool, and add a few drops of strong, but not fuming, nitric acid. Crystals of urea nitrate separate out. Examine these microscopically.

2. **Uric Acid.**—Examine microscopically the crystals of uric acid in some urine, to which 5 per cent. of hydrochloric acid has been added twenty-four hours previously. Note that they are deeply tinged with pigment, and to the naked eye look like granules of cayenne pepper.

Dissolve the crystals in caustic potash and then carefully add excess of hydrochloric acid. Small crystals of uric acid again form.

Place a little uric acid, or a urate (for instance, serpent's urine), in a capsule; add a little dilute nitric acid and evaporate to dryness. A yellowish-red residue is left. Add ammonia. This is best done by holding the capsule upside down over the mouth of a bottle of ammonia. The residue turns to violet. This is due to the formation of murexide or purpurate of ammonia. On the addition of potash the colour becomes bluer.

3. **Deposit of Urates or Lithates (Lateritious Deposit).**—The specimen of urine from the hospital contains excess of urates, which have become deposited on the urine becoming cool. They are tinged with pigment, and have a pinkish colour, like brick-dust; hence the term 'lateritious.' Examine microscopically. The deposit is usually amorphous—that is, non-crystalline. Sometimes crystals of calcium oxalate (envelope crystals—octahedra) are seen.

The deposit of urates dissolves on heating the urine.

4. **Deposit of Phosphates.**—Another specimen of pathological urine contains excess of phosphates, which have formed a white deposit on the urine becoming alkaline. This precipitate does not dissolve on heating; it may be increased. It is, however, soluble in acetic acid. Examine microscopically for coffin-lid crystals of triple phosphate (ammonio-magnesium phosphate), for crystals of stellar (calcium) phosphate, and for mucus. Mucus is flocculent to the naked eye, amorphous to the microscope.

N.B.—On boiling neutral or alkaline urine it may become turbid from deposition of phosphates. The solubility of this deposit in a few drops of acetic acid distinguishes it from albumin, for which it is liable to be mistaken.

Some of the facts described in the foregoing exercises have been already dwelt upon in the preceding lesson. They are, however, conveniently grouped together here, as all involve the use of the microscope.

We have now studied urea, the principal nitrogenous constituent of urine, at some length. There are still left for our consideration a

number of other nitrogenous constituents, the most important of which are uric acid, hippuric acid, and creatinine.

### URIC ACID

**Uric Acid** ( $C_5N_4H_4O_3$ ) is in mammals, next to urea, the medium by which the largest quantity of nitrogen is excreted from the body. It is, however, in birds and reptiles the principal nitrogenous constituent of their urine. It is not present in the free state, but is combined with bases to form urates.)

It may be obtained from human urine by adding 5 c.c. of hydrochloric acid to 100 c.c. of the urine, and allowing the mixture to stand for twelve to twenty-four hours. The crystals which form are deeply tinged with urinary pigment, and though by repeated solution in

caustic soda or potash, and reprecipitation by hydrochloric acid, they may be obtained fairly free from pigment, pure uric acid is more readily obtained from the solid urine of a serpent or bird, which consists principally of the acid ammonium urate. This is dissolved in soda, and then the addition of hydrochloric acid produces as before the crystallisation of uric acid from the solution.

The pure acid crystallises in colourless rectangular plates or prisms. In striking contrast to urea it is a most insoluble substance, requiring for its solution 1900 parts of hot and 15000 parts of cold water. The

forms which uric acid assumes when precipitated from human urine, either by the addition of hydrochloric acid or in certain pathological processes, are very various, the most frequent being the whetstone shape ; there are also bundles of crystal resembling sheaves, barrels, and dumb-bells (see fig. 43).

The murexide test which has just been described among the practical exercises is the principal test for uric acid. The test has received the name on account of the resemblance of the colour to the purple of the ancients, which was obtained from certain snail-like creatures of the genus *Murex*.

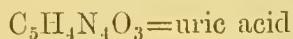
Another important reaction that uric acid undergoes (though it is not applicable as a test) is that on treatment with certain reagents urea and oxalic acid can be obtained from it. The importance of this fact is that probably uric acid may be in the body an antecedent of urea. Excess of uric acid and of oxalic acid generally go together ; and



FIG. 43.—Uric acid crystals.

in deposits of urates, crystals of calcium oxalate are often found as well.

Uric acid is dibasic, and thus there are two classes of urates—the normal urates and the acid urates. A normal urate is one in which two atoms of the hydrogen are replaced by two of a monad metal like sodium; an acid urate is one in which only one atom of hydrogen is thus replaced. The formulae would be—



The acid sodium urate is the chief constituent of the pinkish deposit of urates, which, as we have already stated, is called the lateritious deposit.

The quantity of uric acid excreted by an adult varies from 7 to 10 grains (0.5 to 0.75 grammes) daily.

The best method for determining the quantity of uric acid in the urine is that of Hopkins. Ammonium chloride in crystals is added to the urine until no more will dissolve. This saturation completely precipitates all the uric acid in the form of ammonium urate. The precipitate is collected on a filter and dissolved in weak alkali. From this solution the uric acid is precipitated by neutralising with hydrochloric acid. The precipitate of uric acid is collected on a weighed filter, dried, and weighed.

**Origin of Uric Acid.**—Uric acid is not made by the kidneys. When the kidneys are removed uric acid continues to be formed and accumulates in the organs, especially in the liver and spleen. The liver has been removed from birds, and uric acid is then hardly formed at all, its place being taken by ammonia and lactic acid. It is therefore probable that ammonia and lactic acid are normally synthesised in the liver to form uric acid.

The two conditions which lead to an increase of uric acid in the urine are—

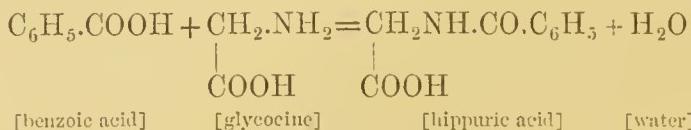
1. Increase of meat diet and diminution of oxidation processes, such as occur in people with sedentary habits.

2. Increase of white corpuscles in the blood, especially in the disease known as leucocythaemia. This latter fact is of great interest, as leucocytes contain large quantities of nuclein. Nuclein yields nitrogenous bases (adenine,  $\text{C}_5\text{H}_5\text{N}_5$ ; hypoxanthine,  $\text{C}_5\text{H}_4\text{N}_4\text{O}$ ) which are closely related to uric acid.

### HIPPURIC ACID

Hippuric acid ( $\text{C}_9\text{H}_9\text{NO}_3$ ), combined with bases to form hippurates, is present in small quantities in human urine, but in large quantities

in the urine of herbivora. This is due to the food of herbivora containing substances belonging to the aromatic group—the benzoic acid series. If benzoic acid is given to a man, it unites with glycocine with the elimination of a molecule of water, and is excreted as hippuric acid—



This is a well-marked instance of synthesis carried out in the

animal body, and experimental investigation shows that it is accomplished by the living cells of the kidney itself; for if a mixture of glycocine, benzoic acid, and blood is injected through the kidney, their place is found to have been taken by hippuric acid.

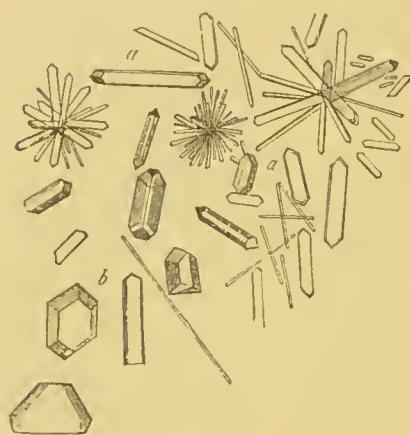


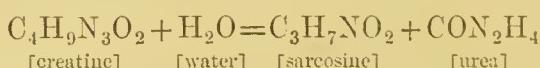
FIG. 44.—Hippuric acid crystals.

the urine even during starvation: this probably represents a small percentage of creatine in the muscles which has escaped decomposition into urea.

The formation of creatinine from creatine is represented in the following equation:—



The small quantity of creatinine in the urine (7 to 10 grains, or 0·5 to 0·9 gramme) will not account for the large quantity of creatine in the muscles (90 grammes). As already stated, it is probably mostly converted into urea. Urea is obtainable artificially from creatine, and it may be that similar reactions occur in the body. The equation that represents the formation of urea from creatine that can be carried out in the laboratory is as follows:—



The two substances formed are urea and sarcosine. Sarcosine is

methyl-glycocine – that is, amido-acetic acid in which one H is replaced by methyl ( $\text{CH}_3$ )



It is not found in the body, and so it is broken up, probably in the liver, forming ammonium carbonate and then urea.

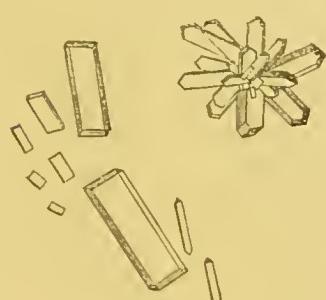


FIG. 45.—Creatine crystals.

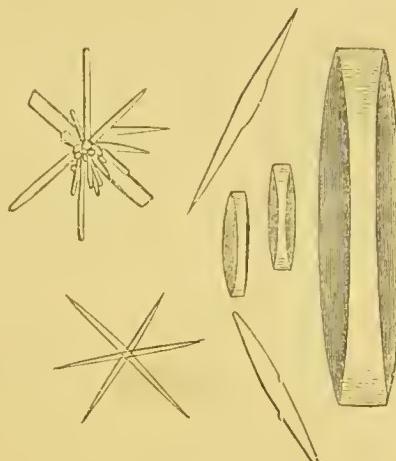


FIG. 46.—Creatinine crystals.

Creatinine with zinc chloride gives a characteristic crystalline precipitate (groups of fine needles) with composition  $\text{C}_4\text{H}_7\text{N}_3\text{O} \cdot \text{ZnCl}_2$ .

### URINARY DEPOSITS

The different substances that may occur in urinary deposits are formed elements and chemical substances.

The **formed or anatomical elements** may consist of blood corpuscles, pus, mucus, epithelium cells, spermatozoa, casts of the urinary tubules, fungi, and entozoa. All of these, with the exception of a small quantity of mucus,<sup>1</sup> which forms a flocculent cloud in the urine, are pathological, and the microscope is chiefly employed in their detection.

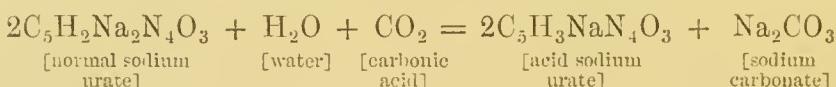
The **chemical substances** are uric acid, urates, calcium oxalate, calcium carbonate, and phosphates. Rarer forms are leucine, tyrosine, xanthine, and cystine. We shall, however, here only consider the commoner deposits, and for their identification must use the microscope and certain chemical tests as well.

**Deposit of Uric Acid.**—This is a sandy reddish deposit resembling cayenne pepper. It may be recognised by its crystalline form (fig. 43, p. 98) and the murexide reaction. The presence of these crystals generally indicates an increased formation of uric acid, and, if excess-

<sup>1</sup> Recent observers state that urinary mucin, like bile mucin, is not true mucin, but a nucleo-albumin.

sive, may lead to the formation of stones or calculi in the bladder. The way in which uric acid is split off from the urates is of great interest to pathologists, and will be found fully discussed in Sir W. Roberts's Croonian Lectures.<sup>1</sup>

**Deposit of Urates.**—This is much commoner, and may, if the urine is concentrated, occur in normal urine when it cools. They are generally found in the concentrated urine of fevers; and there appears to be a kind of fermentation, called the acid fermentation, which occurs in the urine after it has been passed, and which leads to the same result. The chief constituent of the deposit is the acid sodium urate, the formation of which from the normal sodium urate of the urine may be represented by the equation—



This deposit may be recognised as follows :—

1. It has a pinkish colour; the pigment called *uro-erythrin* is derived from the pigment of the urine, but the exact relationship of the two is not known.
2. It dissolves upon warming the urine.
3. Microscopically it is usually amorphous, but crystalline forms similar to those depicted in the next two figures may occur.

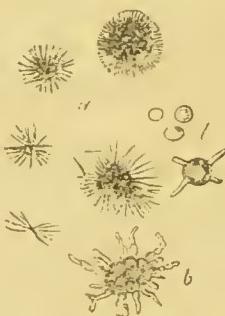


FIG. 47.—Acid sodium urate.

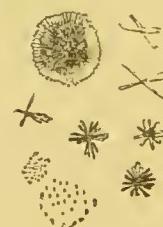


FIG. 48.—Acid ammonium urate.

Crystals of calcium oxalate may be mixed with this deposit (see fig. 49).

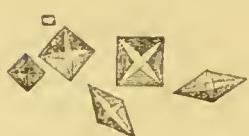


FIG. 49.—Envelope crystals of calcium oxalate.

**Deposit of Calcium Oxalate.**—This occurs in envelope crystals (octahedra) or dumb-bells.

It is insoluble in ammonia, and in acetic acid. It is soluble with difficulty in hydrochloric acid.

**Deposit of Cystin.**—Cystin ( $\text{C}_6\text{H}_{12}\text{N}_2\text{S}_2\text{O}_4$ ) is recognised by its colourless six-sided crystals (fig. 50).

These are rare: they occur only in acid urine, and they may form concretions or calculi.

<sup>1</sup> Published by Smith, Elder, & Co. London, 1892.

**Deposit of Phosphates.**—These occur in alkaline urine. The urine may be alkaline when passed, due to fermentative changes occurring in the bladder. All urine, however, if exposed to the air, will in time become alkaline owing to the growth of the *micrococcus ureæ*. This forms ammonium carbonate from the urea.

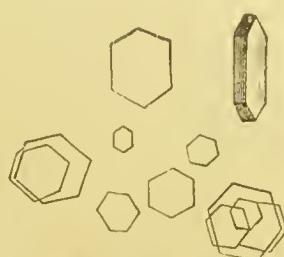
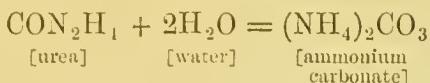


FIG. 50.—Cystin crystals.

The ammonia renders the urine alkaline and precipitates the earthy phosphates. The chief forms of phosphates that occur in urinary deposits are—

1. Calcium phosphate,  $\text{Ca}_3(\text{PO}_4)_2$ ; amorphous.
2. Triple or ammonio-magnesium phosphate,  $\text{MgNH}_4\text{PO}_4$ ; coffin-lids and feathery stars (fig. 51).
3. Crystalline phosphate of calcium,  $\text{CaHPO}_4$ , in rosettes of prisms, in spherules, or in dumb-bells (fig. 52).

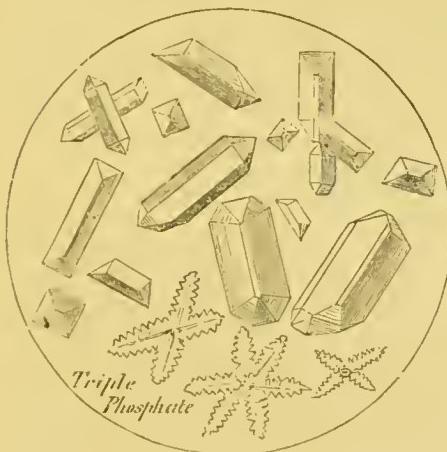


FIG. 51.—Triple phosphate crystals.

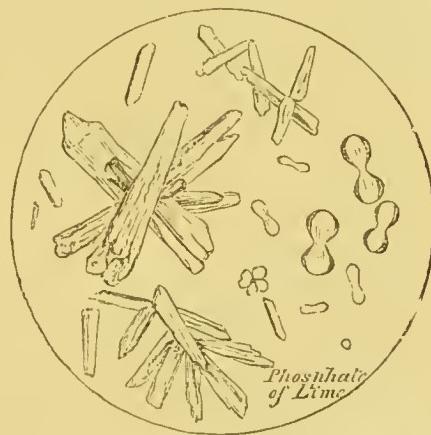


FIG. 52.—Crystals of phosphate of lime (stellar phosphate).

4. Magnesium phosphate,  $\text{Mg}_3(\text{PO}_4)_2 + 22\text{H}_2\text{O}$ , occurs occasionally, and crystallises in long plates.

All these phosphates are dissolved by acids, such as acetic acid, without effervescence.

They do not dissolve on heating the urine; in fact, the amount of precipitate may be increased by heating. Very often neutral or alkaline urine will become cloudy when boiled: this may be due to albumin or to phosphates. It is very important to distinguish between these two, as albuminuria is a serious condition. They may be

distinguished by the use of acetic acid, which dissolves phosphates but not albumin.

A solution of ammonium carbonate (1-in-5) eats magnesium phosphate away at the edges ; it has no effect on the triple phosphate. A phosphate of calcium ( $\text{CaHPO}_4 + 2\text{H}_2\text{O}$ ) may occasionally be deposited in acid urine. Pus in urine is apt to be mistaken for phosphates, but can be distinguished by the microscope.

**Deposit of calcium carbonate**,  $\text{CaCO}_3$ , appears but rarely as whitish balls or biscuit-shaped bodies. It is commoner in the urine of herbivora (see p. 88). It dissolves in acetic or hydrochloric acid, with effervescence.

The following is a summary of the chemical sediments that may occur in urine :—

### CHEMICAL SEDIMENTS IN URINE

#### IN ACID URINE

*Uric Acid*.—Whetstone, dumb-bell, or sheaf-like aggregations of crystals deeply tinged by pigment (fig. 43).

*Urates*.—Generally amorphous. The acid urate of sodium (fig. 47) and of ammonium (fig. 48) may sometimes occur in star-shaped clusters of needles or spheroidal clumps with projecting spines. Tinged brick-red. Soluble on warming.

*Calcium Oxalate*.—Octahedra, so-called envelope crystals (fig. 49). Insoluble in acetic acid.

*Cystin*.—Hexagonal plates (fig. 50). Rare.

*Leucine and Tyrosine*.—Rare.

*Calcium Phosphate*,

$\text{CaHPO}_4 + 2\text{H}_2\text{O}$ .—Rare.

#### IN ALKALINE URINE

*Phosphates*.—Calcium phosphate,  $\text{Ca}_3(\text{PO}_4)_2$ . Amorphous.

Triple phosphate,  $\text{MgNH}_4\text{PO}_4 + 6\text{H}_2\text{O}$ . Coffin-lids or feathery stars (figs. 42 and 51).

Calcium hydrogen phosphate,  $\text{CaHPO}_4$ . Rosettes, spherules, or dumb-bells.

Magnesium phosphate,  $\text{Mg}_3(\text{PO}_4)_2 + 22\text{H}_2\text{O}$ . Long plates.

All soluble in acetic acid without effervescence.

*Calcium Carbonate*,  $\text{CaCO}_3$ .—Biscuit-shaped crystals. Soluble in acetic acid with effervescence.

*Ammonium Urat*,  $\text{C}_5\text{H}_2(\text{NH}_4)_2\text{N}_4\text{O}_3$ .—‘Thorn-apple’ spherules.

*Leucine and Tyrosine*.—Very rare.

## LESSON XII

## PATHOLOGICAL URINE

1. Urine A is pathological urine containing albumin. It gives the usual proteid tests. The two following are most frequently used in practice:—

(a) Boil the top of a long column of urine in a test-tube. If the urine is acid, the albumin is coagulated. If the quantity of albumin is small, the cloudiness produced is readily seen, as the unboiled urine below it is clear. This is insoluble in a few drops of acetic acid, and so may be distinguished from phosphates. If the urine is alkaline, it should be first rendered acid with a little dilute acetic acid.

(b) *Heller's Nitric-acid Test.*—Pour some of the urine gently on to the surface of some nitric acid in a test-tube. A ring of white precipitate occurs at the junction of the two liquids. This test is used for small quantities of albumin.

2. Estimation of Albumin by Esbach's Albuminometer.—Esbach's reagent for precipitating the albumin is made by dissolving 10 grammes of picric acid and 20 grammes of citric acid in 800 or 900 c.c. of boiling water and then adding sufficient water to make up to a litre (1000 c.c.).



FIG. 53.—Albuminometer of Esbach.

Pour the urine into the tube up to the mark U; then the reagent up to the mark R. Close the tube with a cork, and to ensure complete mixture, tilt it to and fro a dozen times without shaking. Allow the corked tube to stand upright twenty-four hours; then read off on the scale the height of the coagulum. The figures indicate grammes of dried albumin in a litre of urine. The percentage is obtained by dividing by 10. Thus, if the coagulum stands at 3, the amount of albumin is 3 grammes per litre, or 0·3 gr. in 100 c.c. If the sediment falls between any two figures, the distance  $\frac{1}{4}$ ,  $\frac{1}{2}$ , or  $\frac{3}{4}$  from the upper or lower figure can be read off with sufficient accuracy. Thus the surface of the sediment being midway between 3 and 4 would be read as 3·5. When the albumin is so abundant that the sediment is above 4, a more accurate result is obtained by first diluting the urine with one or two volumes of water, and then multiplying the resulting figure by 2 or 3, as the case may be. If the amount of albumin is less than 0·5 per cent., it cannot be accurately estimated by this method.

3. Urine B is diabetic urine. It has a high specific gravity. The presence of sugar is shown by the reduction (yellow precipitate of cuprous oxide) that occurs on boiling with Fehling's solution. Fehling's solution is an alkaline solution of copper sulphate to which Rochelle salt has been added. The Rochelle salt (double tartrate of potash and soda) holds the cupric hydrate in solution. Fehling's solution should always be freshly prepared, as, on standing, racemic acid is formed from the tartaric acid, and this substance itself reduces the cupric to cuprous oxide. Fehling's solution should, therefore, always be tested by boiling before it is used. If it remains clear on boiling, it is in good condition.

4. Quantitative Determination of Sugar in Urine.—Fehling's solution is prepared as follows:—34·639 grammes of copper sulphate are dissolved in about 200 e.e. of distilled water; 173 grammes of Rochelle salt are dissolved in 600 c.c. of a 14-percent. solution of caustic soda. The two solutions are mixed and diluted to a litre. Ten c.c. of this solution are equivalent to 0·05 grammie of dextrose. Dilute 10 e.e. of this solution with about 40 e.e. of water, and boil it in a flask. Run into this from the burette (see fig. 54) the urine (which should be previously diluted with nine times its volume of distilled water) until the blue colour of the copper solution disappears—that is, till all the cuprie hydrate is reduced.<sup>1</sup> The mixture in the flask should be boiled after every addition.<sup>2</sup> The quantity of diluted urine used from the burette contains 0·05 grammie of sugar. Calculate the percentage from this, remembering that the urine has been diluted to ten times its original volume.

The following formula will be found useful in converting grammes into grains:—

$$x = \text{number of grains of sugar in the 24 hours.}$$

$$a = \text{number of ounces of urine in the 24 hours.}$$

$$1 \text{ ounce} = 28\cdot396 \text{ c.c.}$$

$b$  = number of e.e. of urine used from the burette to decompose 10 e.e. of Fehling's solution (equivalent to 0·05 grammie = 0·77 grain of sugar).

Then

$$x = \frac{a}{b} \times 28\cdot396 \times 0\cdot77 = \frac{a}{b} \times 21\cdot865.$$

FIG. 54.—Two burettes on stand. (Sutton.)

The full significance and cause of pathological urine cannot be appreciated until a theoretical and practical acquaintance with disease is obtained, and we shall briefly consider only those abnormal constituents which are most frequently met with.

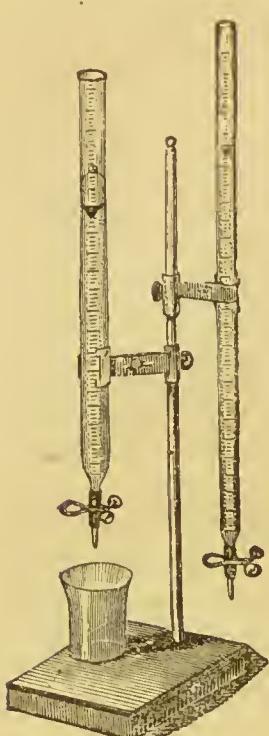
### PROTEIDS IN THE URINE

There is no proteid matter in normal urine, and the most common cause of the appearance of albumin in the urine is disease of the kidney (Bright's disease). The best methods of testing for and estimating the albumin are given in the practical heading to this lesson. The term 'albumin' is the one used by clinical observers. Properly speaking, it is a mixture of serum albumin and serum globulin.

A condition called 'peptonuria,' or peptone in the urine, is observed in certain pathological states, especially in diseases where there is a formation of pus, and particularly if the pus is decomposing owing

<sup>1</sup> It is somewhat difficult for the unpractised observer to determine accurately the exact point at which the blue disappears. The blue colour, if any remains, will be seen by holding the flask up to the light. Some prefer a white porcelain basin instead of a flask; the blue can then be seen against the white of the basin.

<sup>2</sup> On cooling the blue colour reappears, owing to reoxidation.



to the action of a bacterial growth called staphylococcus ; one of the products of the disintegration of pus cells appears to be peptone ; and this leaves the body by the urine. The term 'peptone,' however, includes the 'proteoses.' Indeed, in most cases of so-called peptonuria, true peptone is absent. In the disease called 'osteomalacia' a proteose is usually found in the urine.

### SUGAR IN THE URINE

Normal urine contains no sugar, or so little that observers are not agreed whether it is sugar or not. It occurs in the disease called diabetes mellitus, which can be artificially produced by puncture of the medulla oblongata, or by extirpation of the pancreas. The disease as it occurs in man may be due to disordered metabolism of the liver, to disease of the pancreas, or to other not fully understood causes.<sup>1</sup>

The methods usually adopted for detecting and estimating the sugar are given at the head of this lesson. The sugar present is dextrose. Lactose may occur in the urine of nursing mothers. Diabetic urine also contains hydroxybutyric acid, and may contain or yield on distillation acetone and ethyl-diacetic acid.

Fehling's test is not absolutely trustworthy. Often a normal urine will decolorise Fehling's solution, though seldom a red precipitate is formed. This appears to be due to excess of urates and creatinine. Another substance called glycuronic acid ( $C_6H_{10}O_7$ ) is, however, very likely to be confused with sugar by Fehling's test ; the cause of its appearance is sometimes the administration of drugs (chloral, camphor, &c.) ; but sometimes it appears independently of drug treatment. The cause of this is not known, but the condition has not the serious meaning one attaches to diabetes ; hence, for life assurance purposes, it is most necessary to confirm the presence of sugar by other tests.

Then, too, in the condition called alcaptonuria, confusion may similarly arise. Alcapton is a substance which probably originates from tyrosine by an unusual form of metabolism. It gives the urine a brown tint, which darkens on exposure to the air. It is an aromatic substance, and the recent researches of Baumann and Wolkow<sup>2</sup> have identified it with homogentisic acid ( $C_6H_3(OH)_2CH_2COOH$ ).

The best confirmatory test for sugar is the *fermentation test*, which is performed as follows :—

Half fill a test tube with the urine and add a little German yeast. Fill up the tube with mercury ; insert it in a basin of mercury, and leave it in a warm place for twenty-four hours. The sugar will undergo fermentation ; carbonic acid gas accumulates in the tube, and the liquid no longer gives the tests for sugar, or only faintly, but gives those for alcohol instead. A control experiment should be made with yeast and water in another test-tube, as a small yield of carbonic acid is sometimes obtained from impurities in the yeast.

<sup>1</sup> Transitory glycosuria is found in many diseases.

<sup>2</sup> *Zeit. physiol. Chem.* xv. p. 228.

### BILE IN THE URINE

This occurs in jaundice. The urine is dark-brown, greenish, or in extreme cases almost black in colour. The most readily applied test is Gmelin's test for the bile pigments. Pettenkofer's test for the bile acids seldom succeeds in urine if the test is done in the ordinary way. The best method is to warm a thin film of urine and cane sugar solution in a flat porcelain dish. Then dip a glass rod in strong sulphuric acid, and draw it across the film. Its track is marked by a purplish line. Excess of urobilin should not be mistaken for bile pigment.

### BLOOD AND BLOOD PIGMENT IN THE URINE

When haemorrhage occurs in any part of the urinary tract, blood appears in the urine. It is found in the acute stage of Bright's disease. If a large quantity is present, the urine is deep red. Microscopic examination reveals the presence of blood corpuscles, and on spectroscopic examination the bands of oxyhaemoglobin are seen.

If only a small quantity of blood is present, the secretion—especially if acid—has a characteristic reddish-brown colour, which physicians term 'smoky.'

The blood pigment may under certain circumstances appear in the urine without the presence of any blood-corpuscles at all. This is produced by a disintegration of the corpuscles occurring in the circulation, and the most frequent cause of this is a disease allied to ague, which is called *paroxysmal haemoglobinuria*. The pigment is in the condition of methaemoglobin mixed with more or less oxyhaemoglobin, and the spectroscope is the means used for identifying these substances (see p. 80).

### PUS IN THE URINE

Pus occurs in the urine as the result of suppuration in any part of the urinary tract. It forms a white sediment resembling that of phosphates, and, indeed, is frequently mixed with phosphates. The pus corpuscles may, however, be seen with the microscope; their nuclei are rendered evident by treatment with 1-per-cent. acetic acid, and the pus corpuscles are seen to resemble white blood corpuscles, which, in fact, they are in origin.

On the addition of liquor potassæ to the deposit of pus cells aropy gelatinous mass is obtained. This is distinctive. Mucus treated in the same way is dissolved. Some of the proteid constituents of the pus cells—and the same is true for blood—pass into solution in the urine, so that the urine pipetted off from the surface of the deposit gives the tests for albumin.

## DETECTION OF PHYSIOLOGICAL PROXIMATE PRINCIPLES

Subsequent lessons may be very usefully employed by the class in testing for the various substances the properties of which they have studied. The following scheme will form a rough guide to the tests to be employed :—

1. Note reaction, colour, clearness or opalescence, taste, smell.
2. Coloured liquids suggest blood, bile, urinary pigment, &c.
3. Opalescent liquids contain starch, glycogen, or certain proteids.
4. Add iodine.

Coloration produced :

**Blue:** Starch. Confirm by converting into sugar by saliva at 40° C., or boiling with dilute sulphuric acid.

Reddish brown: Glycogen or dextrin. Glycogen forms an opalescent solution in water, and readily precipitates by alcohol. It is precipitated by basic lead acetate. Dextrin forms a clear solution : it is not precipitated by basic lead acetate unless ammonia is added also. It is not precipitated by alcohol unless a large excess is added. Both dextrin and glycogen are like starch convertible into sugar.

5. Add copper sulphate and caustic potash.
- (a) Blue solution : boil ; yellow or red precipitate. Dextrose, maltose, or lactose (for distinguishing tests see Lesson XIII.).
- (b) Blue solution ; very slight reduction on boiling ; boil some of the original solution with dilute sulphuric acid, and then boil with copper sulphate and caustic potash ; abundant yellow or red precipitate: Cane sugar.
- (c) Violet solution : Proteids (albumins, globulins, albuminates). In presence of magnesium sulphate the potash causes also a white precipitate of magnesia.

(d) Pink solution ; biuret reaction. Peptones or albumoses (proteoses). In presence of ammonium sulphate very large excess of potash is necessary for this test. Only a trace of copper sulphate must be used.

6. When proteids are present proceed as follows : Boil the original solution (after adding a trace of 2-per-cent. acetic acid).

- (a) Precipitate produced : Albumins or globulins.
- (b) No precipitate : Albuminates, proteoses, or peptones.

7. If albumin or globulin is present, saturate a fresh portion with magnesium sulphate ; filter ; the precipitate contains the globulin, the filtrate the albumin. Test temperature of heat coagulation.

8. If albumin or globulin is absent :—

(a) Neutralisation causes a precipitate soluble in excess of weak acid or alkali. Acid-albumin or alkali-albumin, according as the reaction of the original liquid is acid or alkaline respectively. If the original liquid is neutral, acid-albumin and alkali-albumin must be both absent.

(b) Neutralisation produces no such precipitate : **Proteose** or **peptone**.

9. If proteose, or peptone, or both, are present, saturate a fresh portion with ammonium sulphate :

(a) Precipitate : **Proteose**. (b) No precipitate : **Peptone**.

If both are present, the precipitate contains the proteose, and the filtrate the peptone.

10. To a fresh portion add nitric acid (proteids having been proved to be present).

(a) No precipitate, even though excess of sodium chloride be also added : **Peptone**.

(b) No precipitate, until excess of sodium chloride is added : **Deutero-proteose**.

(c) Precipitate which disappears on heating and reappears on cooling : **Proteoses**. This is the distinctive test of all the proteoses or albumoses, and is given by all of them. For one of them, however (deutero-proteose), excess of sodium chloride must be added also.

(d) Precipitate little altered by heating : **Albumin** or **globulin**.

In all four cases nitric acid *plus* heat causes a yellow colour, turned orange by ammonia.

11. Confirmatory tests for proteids :—

(a) Millon's test.

(b) Ferrocyanide of potassium and acetic acid causes a precipitate (except in the case of peptones and some proteoses).

(c) To test for fibrinogen :—

i. It coagulates by heat at 56° C.

ii. It is changed into fibrin by fibrin ferment and calcium chloride.

(d) To test for caseinogen :—

i. It is not coagulated by heat.

ii. It is changed into casein by rennet and calcium chloride.

12. If **blood** is suspected—

(a) Examine spectroscopically, diluting if necessary.

i. Oxyhaemoglobin shows two bands between D and E.

ii. Add ammonium sulphide ; one band only appears.

iii. Carbonic oxide haemoglobin shows two bands also, but will not reduce with ammonium sulphide.

iv. Methaemoglobin gives a typical band in the red between C and D.

v. Haematin &c. show special spectra (see Lesson XX.).

(b) Dry ; boil with glacial acetic acid and a crystal of sodium chloride on a glass slide under a cover glass. Haemin crystals are obtained.

(c) If the blood is old and dry, and its haemoglobin converted into haematin :

i. Try haemin test.

ii. Dissolve it in potash ; add ammonium sulphide, and examine for spectrum of haemochromogen (see Lesson XX.).

13. If bile is suspected—

- (a) Try Gmelin's test for bile pigments. This is the test for bile in urine.
- (b) Try Pettenkofer's test for bile-salts.

14. Miscellaneous substances :

(a) **Mucin.** Precipitated by acetic acid or by alcohol. The precipitate is soluble in lime water. By collecting the precipitate and boiling it with 25-per-cent. sulphuric acid, a reducing sugar is obtained. Mucin gives the proteid colour tests.

(b) **Gelatin.** This also gives the proteid colour tests. It is not coagulated, but dissolved in hot water. The solution gelatinises when cold.

(c) **Urea.** Very soluble in water. The solution effervesces when sodium hypobromite or fuming nitric acid are added. Concentrate a fresh portion, add nitric acid, and examine for crystals of urea nitrate.

(d) **Uric acid.** Very insoluble in water; soluble in potash, and precipitated from this solution in crystals by hydrochloric acid. Uric acid crystals from human urine are deeply pigmented red. Try murexide test.

(e) **Cholesterin.** Characteristic flat crystalline plates. Play of colours with iodine and concentrated sulphuric acid.

15. Urine. Normal constituents :

(a) **Chlorides.** Acidulate with nitric acid ; add silver nitrate ; white precipitate.

(b) **Sulphates.** Acidulate with hydrochloric acid ; add barium chloride ; white precipitate.

(c) **Phosphates.** Acidulate with nitric acid ; add ammonium molybdate ; boil ; and a yellow crystalline precipitate forms. To another portion add ammonia ; earthy (*i.e.* calcium and magnesium) phosphates are precipitated.

(d) **Urea** (see above).

(e) **Uric acid.** To 100 c.c. of urine add 5 c.c. of hydrochloric acid ; leave for twenty-four hours, and pigmented crystals of uric acid are formed. For tests see above.

(f) **Hippuric acid.** Evaporate the urine with nitric acid, and heat the residue in a dry test-tube. A smell of oil of bitter almonds is given off.

(g) **Creatinine.** Take 250 c.c. of urine ; add milk of lime and calcium chloride in excess to precipitate phosphates ; filter, and evaporate filtrate to a small bulk ; to this add 50 c.c. absolute alcohol, and let the mixture stand six hours ; add 10 to 15 drops of an alcoholic solution of zinc chloride. Crystals (rosettes) of creatinine zinc chloride form in the course of a day or two.

16. Urine. Abnormal constituents.

(a) **Blood.** Microscope (blood corpuscles). Spectroscope (for oxyhaemoglobin or methaemoglobin). Haemin test.

(b) **Blood pigment** may be present without blood corpuscles. Spectroscope.

(c) **Bile.** Gmelin's test.

(d) *Pus.* White deposit. Microscope (pus cells). Add potash ; it becomes stringy.

(e) *Albumin.* (i.) Precipitated, if acid, by boiling ; precipitate insoluble in acetic acid, so distinguishing it from phosphates. (ii.) Precipitated by nitric acid in the cold.

(f) *Sugar.* (i.) Brown colour with potash and heat (Moore's test). (ii.) Ferments with yeast. (iii.) Reduces Fehling's solution. (iv.) Urine has a high specific gravity.

(g) *Mucus.* Flocculent cloud ; may be increased by acetic acid ; soluble in alkalis. A little mucus in urine is not abnormal.

(h) *Deposits.*

i. Examine microscopically for blood corpuscles, pus cells, crystals, &c.

ii. *Phosphates.* White deposit often mixed with mucus or pus. Insoluble on heating ; soluble in acetic acid. Urine generally alkaline. Examine microscopically for coffin-lids of triple phosphate and star-like clusters of stellar (calcic) phosphate.

iii. *Uric acid.* Like cayenne pepper. Whetstone &c. crystals. Very insoluble in water. Soluble in potash. Murexide test. Urine acid.

iv. *Urates.* Pink deposit, usually amorphous ; may be mixed with envelope crystals of calcium oxalate. Deposit soluble on heating urine. Murexide test.

## ADVANCED COURSE



### INTRODUCTION

IT will be presupposed that students who take the following lessons have already been through the elementary course. The order in which the subjects are treated is the same as that already adopted. The instructions given will be mainly practical; the theoretical matter on which they depend, or to which they lead, is, as a rule, too lengthy to be discussed in a short manual like the present volume. References are therefore given to the author's larger work on chemical physiology. The Appendix contains a description of various instruments which are not generally contained in sufficient numbers in a physiological laboratory to admit of each student being able to use them in a class. It also contains a description of certain methods of research which should always be shown in demonstrations, though there may be practical difficulties in allowing each member of the class to perform the experiments.

The few experiments in which living animals are employed will also necessarily be of the nature of demonstrations.

## LESSON XIII

## CARBOHYDRATES

1. **Glycogen.**—A rabbit which has been fed five or six hours previously on carrots is killed by bleeding. The chest and abdomen are opened quickly, and a cannula inserted into the portal vein, and another into the vena cava inferior. A stream of salt solution is then allowed to pass through the liver until it is uniformly pale. The washings are collected in three beakers labelled *a*, *b*, and *c*.

The liver is cut out quickly, chopped into small pieces, and thrown into boiling water acidulated with acetic acid. The acidulated water extracts a

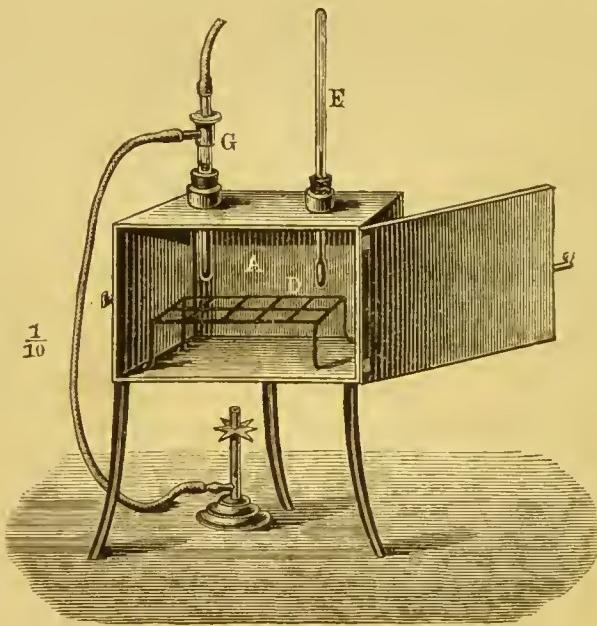


FIG. 55.—Hot-air oven with gas regulator (*G*). (Gseheidlen.)

small quantity of glycogen. The pieces of sealed liver are then ground up in a mortar with hot water, and thoroughly extracted with boiling water. Filter. A strong solution of glycogen is thus obtained.

Test the solution with iodine.

To separate the glycogen<sup>1</sup> evaporate the solution to a small bulk on the water-bath and add excess of alcohol; the glycogen is precipitated as a

<sup>1</sup> This method of preparation of glycogen has the advantage that only traces of protein are mixed with it. In Kühl's method (extraction with dilute potash) there is more protein. This is precipitated by the alternate addition of hydrochloric acid and potassium-mercuric iodide.

flocculent powder, which is collected on a filter and dried in an oven at the temperature of  $100^{\circ}$  (see fig. 55).

If the experiment is to be a quantitative one, the piece of liver taken and the glycogen obtained must be weighed.

2. Examine the washings of the liver in the beakers *a*, *b*, and *c* for sugar. This may be done in a rough quantitative manner as follows:—Take equal quantities of *a*, *b*, and *c* in three test-tubes; to each add an equal amount of Fehling's solution, and boil: *a* will give a heavy precipitate, *b* one not so heavy, and *c* least of all, or none at all.

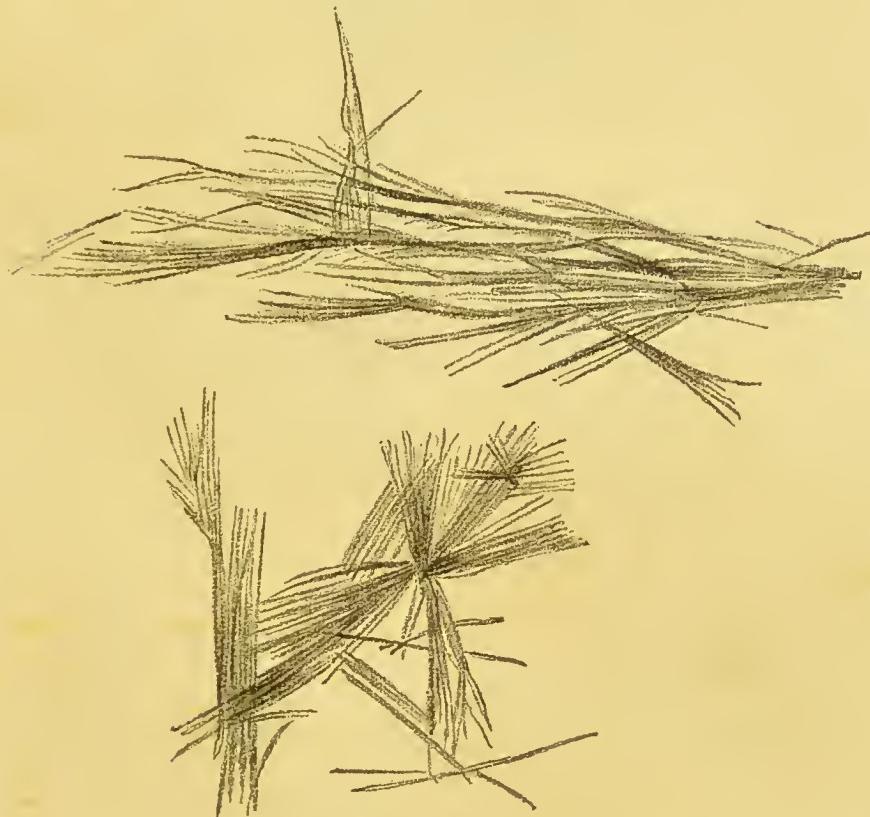


FIG. 56.—Phenyl-glucosazone  $\times 120$ .

3. Phenyl Hydrazine Test for Sugars.—To 50 c.c. of the suspected fluid (*e.g.* diabetic urine) add 1 to 2 grammes of phenyl hydrazine hydrochloride, 2 gr. of sodium acetate, and heat on the water-bath for half an hour. On cooling, if not before, a crystalline or amorphous precipitate separates out. If amorphous, dissolve it in hot alcohol; dilute the solution with water, and boil to expel the alcohol, whereupon the compound or osazone separates out in yellow crystals.

Examine the crystals with the microscope. Dextrose gives a precipitate of phenyl-glucosazone ( $C_{18}H_{22}N_4O_4$ ), which crystallises in yellow needles (melting-point  $205^{\circ}$  C.). Levulose yields an osazone identical with this.

Galactose yields a very similar osazone (phenyl-galactosazone). It differs

from phenyl-glucosazone by melting at 190–193°, and in being optically inactive when dissolved in glacial acetic acid. Cane sugar does not form a compound with phenyl-hydrazine.

Maltose yields phenyl-maltosazone ( $C_{24}H_{32}N_4O_9$ ). It crystallises in yellow needles much wider than those yielded by glucose or lactose (melting-point 205° C.). Unlike phenyl-glucosazone it dissolves in 75 parts of boiling water, and is still more soluble in hot alcohol.

Lactose yields phenyl-lactosazono ( $C_{24}H_{32}N_4O_9$ ). It crystallises readily

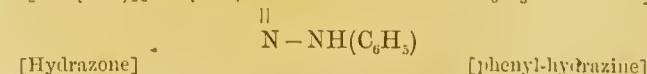
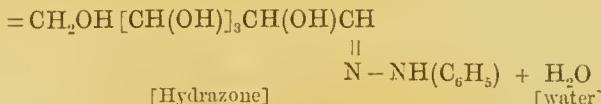


FIG. 57.—Phenyl-lactosazono  $\times 120$ .

in needles, usually in clusters (melting-point 200° C.). It is soluble in 80–90 parts of boiling water.<sup>1</sup>

**4. Barfoed's Reagent.**—Dissolve 1 part of cupric acetate in 15 parts of water; to 200 c.c. of this solution add 5 c.c. of acetic acid containing 38 per

<sup>1</sup> The chemistry of the reactions is represented in the following equations, dextrose being taken as an example of the sugar used :—



cent. of glacial acetic acid. Dextrose reduces this reagent on boiling ; maltose and lactose do not.

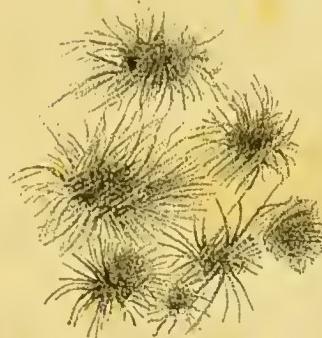


FIG. 58.—Phenyl-maltosazone  $\times$  120.

**5. The Polarimeter.**—Estimate the strength of a solution of dextrose by means of the polarimeter (see Appendix).

(See ‘Chemical Physiology and Pathology’; Glycogen, pp. 539–550; Osazones, p. 110; Polarimeter, pp. 36–45.)

## LESSON XIV

### *ACTION OF MALT UPON STARCH. MALTOSE*

1. Prepare a  $0\frac{7}{10}$  solution of starch.
2. Prepare some malt extract by digesting 10 grammes of powdered malt with 50 c.c. of water at  $50^{\circ}\text{ C}.$  for three hours, and subsequently straining. This extract contains the diastatic or malting ferment.
3. To the starch solution add one-tenth of its volume of malt extract, and place the mixture in a water-bath at  $40^{\circ}\text{ C}.$  From time to time test portions of the liquid by mixing a drop with a drop of iodine solution on a testing slab. The blue colour at first seen is soon replaced by a violet (mixture of blue and red), and then by a red reaction (due to *erythro-dextrin*) which gradually vanishes. Alcohol added to the liquid when all starch and erythro-dextrin have gone still causes a precipitate of a dextrin, which, as it gives no colour with iodine, is called *achroo-dextrin*. The liquid also contains a reducing sugar, maltose.
4. Treat the solution of starch as before with one-tenth of its volume of malt extract, and keep it in the warm chamber ( $50^{\circ}\text{ C}.$ ) for three hours. This may be conveniently done beforehand by the demonstrator.
5. Take 50 c.c. of the product (which is a solution of maltose) and determine how much of it is necessary to reduce 10 c.c. of Fehling's solution. The manner of carrying out Fehling's quantitative method is given in Lesson XII.
6. Take another 50 c.c. and boil it with 1 c.c. of strong sulphuric acid for half an hour in a flask. This converts it into dextrose. After cooling bring the liquid to its original volume (50 c.c.) by adding water and again determine its increased reducing power with Fehling's solution. If  $x = \text{c.c.}$  of maltose solution necessary to reduce 10 c.c. of Fehling's solution, then  $\frac{2x}{3} = \text{c.c.}$  of dextrose solution necessary for the same purpose. The strength of the maltose solution can be calculated from the fact that 10 c.c. of Fehling's solution corresponds to 0.05 gr. of dextrose.

(See 'Chemical Physiology and Pathology,' pp. 103-106, 814, 815.)

## LESSON XV

*COAGULATION OF MILK*

1. Prepare a solution of rennet by extracting the fourth stomach of the sucking calf with glycerine. Clark's rennet, which is sold for making junkets, will do equally well.

2. Prepare a solution of pure caseinogen in the following way:—Saturate milk with magnesium sulphate by shaking it with excess of the powdered salt. Allow it to stand for a few hours, and then filter. The caseinogen and fat remain together on the filter. Save the filtrate, and label it A. Wash the precipitate on the filter with saturated solution of magnesium sulphate until the washings contain no albumin. Add water to the precipitate. The caseinogen dissolves, the fat being insoluble. In this way a solution of caseinogen in weak magnesium sulphate is obtained. So far the operations should be performed beforehand by the demonstrator.

3. To this solution add acetic acid. The caseinogen is precipitated; collect it on a filter; wash the acid away with distilled water. Dissolve the precipitate in lime water by grinding it up in a mortar with the lime water; filter, and an opalescent solution of caseinogen is obtained.

4. To a portion of this solution add a few drops of rennet extract. Put it in the water-bath at 40° C., and if the caseinogen has been thoroughly washed no coagulation will occur.

5. Treat another portion in the same way, adding, however, a few drops of 0·5-per-cent. phosphoric acid as well as the rennet. Warm to 40° C. Coagulation—that is, formation of casein from caseinogen—usually occurs in a few minutes.

6. Examine the filtrate A (see above). Saturate a portion with sodium chloride. A small amount of precipitate of a proteid comes down. This is the so-called lacto-globulin. This contains only a trace of true globulin: it is mostly caseinogen previously left in solution, together with calcium sulphate.

7. Heat another portion of A to 77°, acidifying faintly with a few drops of 2-per-cent. acetic acid. Lactalbumin is coagulated at this temperature.

8. Ringer's method of showing the conversion of caseinogen into casein:—Milk is strongly acidified with acetic acid. This precipitates the caseinogen and entangled fat. The precipitate is collected on a filter, thoroughly washed with distilled water, and ground up in a mortar with calcium carbonate. The mixture is thrown into excess of distilled water. The fat rises to the

top; the excess of calcium carbonate falls to the bottom. The intermediate fluid contains the caseinogen in solution; it is usually very opalescent. Take some of this solution and divide it into three parts, A, B, and C.

To A add rennet.

To B add a few drops of 10-per-cent. solution of calcium chloride.

To C add both rennet and calcium chloride.

Put all three in the water-bath at 40° C. A clot of casein forms in C, but not in A and B.

(See 'Chemical Physiology and Pathology,' pp. 580-585.)

B & A ~~caseinogen~~ clot forms.

## LESSON XVI

*FLOUR*

A. Prepare some gluten by washing a quantity of flour in a muslin bag (see Lesson IV.). It is sticky and elastic. Treat small portions as follows :—

1. Add 10-per-cent. sodium chloride solution ; no gluten dissolves. The liquid may become slightly turbid if all the starch has not been washed away from the gluten.

2. Grind up some with alcohol in a mortar. Filter. The filtrate is opalescent, it having dissolved out a proteose from the gluten. The residue is not sticky, and is called gluten-fibrin. Test the filtrate as follows :—Add distilled water ; this gives a white precipitate. Boiling gives no precipitate. A trace of copper sulphate and caustic potash give a pink colour (biuret reaction).

B. Flour has been extracted with 10-per-cent. sodium chloride solution. After twenty-four hours the supernatant fluid is siphoned off. This extract has the following characters :—

1. Reaction is neutral.

2. Iodine gives a reddish-brown colour due to erythro-granulose.

3. Copper sulphate and potash give a violet colour.

4. Magnesium sulphate added to saturation gives a dense precipitate of globulin and proteose.

5. Saturation with sodium chloride also gives a precipitate. This is less dense, and consists chiefly of globulin. Filter. The filtrate gives a precipitate of proteose with acetic acid.

6. Boil the original liquid. Globulin is coagulated. Filter. The filtrate contains the proteose, and gives the following tests :—(a) Biuret reaction. (b) Saturation with magnesium sulphate or sodium chloride gives a precipitate ; the precipitate with sodium chloride is, however, not very dense unless acetic acid be added also. (c) Acetic acid gives a precipitate. (d) Nitric acid gives a precipitate in the cold, which dissolves on heating and reappears on cooling.

C. Examine the residue of the flour after extraction with 10-per-cent. sodium chloride solution. Wash it in a muslin bag and observe that very little gluten is obtainable, as the proteids, which are the precursors of gluten, have been previously extracted by the salt solution.

(For Vegetable Proteids see ‘Chemical Physiology and Pathology,’ pp. 131–137.)

## LESSON XVII

*THE ALBUMOSES*

1. Witte's peptone contains very little true peptone, but consists chiefly of albumoses, which are soluble, like peptone, in neutral saline solutions.

2. Make a solution of this substance in 10-per-cent. sodium chloride solution, and filter. Very little residue is left on the filter. This consists of dysalbumose, an insoluble form of hetero-albumose, formed during the process of preparing the substance. If hot saline solution is used instead of cold as a solvent, this amount of insoluble residue is increased, hetero-albumose being to a slight extent precipitated by heat.

3. The solution gives the following tests :—

(a) Biuret reaction (due both to peptone and albumoses).

(b) A drop of nitric acid gives a precipitate, which dissolves upon heating and reappears on cooling. (This is due to the albumoses present.)

(c) It does not coagulate on heating.

Otherwise it gives the ordinary proteid reactions.

4. For the separation of the albumoses and peptone proceed as follows :—

(a) Saturate the solution with ammonium sulphate, and filter. The filtrate contains the peptone and the precipitate the albumoses. The peptone is not precipitated by nitric acid, nor by most of the reagents that precipitate other proteids. It is precipitated completely by alcohol, tannin, and potassium-mercuric iodide; imperfectly by phospho-tungstic and phospho-molybdic acid. It gives the biuret reaction, but in the presence of ammonium sulphate a large excess of caustic potash is necessary.

(b) Dialyse another portion of the solution; hetero-albumose is precipitated.

(c) Saturate another portion of the solution with sodium chloride after faintly acidulating with acetic acid. Proto-albumose and hetero-albumose are precipitated. Filter. The filtrate contains the deutero-albumose and peptone.

The proto- and hetero-albumose may be redissolved by adding distilled water, and may be separated from each other by dialysis (see b).

Deutero-albumose may be separated from the peptone by saturation with ammonium sulphate, or by the addition of a crystal of phosphoric acid. These reagents precipitate the deutero-albumose, but not the peptone.

Deutero-albumose gives the nitric-acid reaction (see 3, b) characteristic of the albumoses only in the presence of excess of salt. If the salt is removed by dialysis, nitric acid then causes no precipitate.

(See 'Chemical Physiology and Pathology,' pp. 128-131 and 645-648.)

## LESSON XVIII

*DIGESTION*

1. Examine the comparative digestive power of the glycerine extracts of two stomachs. Take in two test-tubes an equal small weighed quantity of fibrin stained with carmine. Add to each 10 c.c. of 0·2 per cent. hydrochloric acid. Add to one a measured quantity of one glycerine extract, and to the other an equal quantity of the other glycerine extract. As the fibrin is digested the carmine is set free, and colours the liquid; that which is more deeply stained is that which contains the more active preparation of pepsin.

2. Pancreatic digestion. A finely divided ox-pancreas has been allowed to digest at 40° C. for twenty-four to thirty-six hours in a litre of 1 per cent. sodium carbonate to which the white of an egg has been added every ten hours. Note the odour, due to putrefaction. Another preparation has been similarly made except that thymol has been added to prevent decomposition. These should be got ready by the demonstrator.

3. Filter some of the extract and examine for leucine and tyrosine as follows:—

(a) To some of the liquid add Millon's reagent and filter off the precipitated proteid. Boil the filtrate. The presence of tyrosine is indicated by a red colour. If tyrosine is abundant the red colour appears without boiling.

(b) Boil another portion of the filtrate; filter off the proteid thus coagulated; reduce the filtrate to a small bulk by evaporation on the water-bath at the boiling temperature. Examine a drop microscopically for crystals of leucine and tyrosine. Treat the remainder with excess of alcohol to precipitate the albumoses and peptones and again filter. Concentrate the filtrate on the water-bath till it becomes sticky from the presence of leucine. Examine some of the concentrated fluid with the microscope; leucine will be found in crystalline spheroidal clumps.

4. Examine microscopic specimens of leucine and tyrosine which have been prepared by the demonstrator.

The first of these additional exercises on digestion illustrates the principle of Grützner's method of comparing the digestive powers of solutions. In the original method the amount of carmine set free is estimated by an artificial scale consisting of ten solutions of carmine of different known strengths.

The carmine solution for staining the fibrin is prepared by dissolving 1 grammie of carmine in about 1 c.c. of ammonia; to this 400 c.c. of water

are added, and the mixture is kept in a loosely stoppered bottle till the smell of ammonia has become faint.

The fibrin is stained by taking it perfectly fresh and clear. It is chopped fine and placed in the carmine solution for twenty-four hours. The fluid is strained off and the fibrin washed in water till the washings are colourless. It is kept in a stoppered bottle with just enough ether to cover it.

(See 'Chemical Physiology,' pp. 645, 654-663.)

### ADDITIONAL EXERCISES

Among the important reactions of proteids is Piotrowski's reaction—that is, the coloration produced by copper sulphate and a caustic alkali; the term 'biuret reaction' is applied to the rose-red colour which proteoses and peptones give with these reagents, because biuret (a derivative of urea) gives a similar colour. It does not, however, prove that biuret is contained in the proteid molecule. Biuret and proteid both contain some radicle to which the colour is due. Gnezda<sup>1</sup> thought it was cyanogen, and that the cyanogen was differently combined in the peptones and native proteids (albumins and globulins) respectively; hence the rose-red given by one group and the violet by the other. More recent work by Pickering,<sup>2</sup> however, points to a CONH group rather than cyanogen. Gnezda found that if a dilute solution of nickel sulphate is used instead of copper sulphate, the native proteids give different colours from the peptones and proteoses, and Pickering has found the same with cobalt. Their results may be given in the following table :—

Proteid	Copper sulphate and ammonia	Copper sulphate and potash	Nickel sulphate and ammonia	Nickel sulphate and potash	Cobalt sulphate and ammonia	Cobalt sulphate and potash
Albumins and globulins }	Blue	Violet	Nil	Yellow	Nil	Heliotrope-purple
Proteoses and peptones }	Violet	Rose-red	Yellow	Orange	Nil	Red-brown

Another delicate test recently introduced by McWilliam may here be mentioned: Salicyl-sulphonic acid precipitates albumins and globulins; on heating the precipitate is coagulated. The same reagent precipitates proteoses. On heating the precipitate dissolves and reappears on cooling. It does not precipitate peptones.

### Tests for Free Hydrochloric Acid

(a) Gunsberg's reagent consists of 2 parts of phloroglucinol, 1 part of vanillin, and 30 parts of rectified spirit. A drop of filtered gastric juice

<sup>1</sup> Proc. Roy. Society, vol. xlvi. p. 202.

<sup>2</sup> Journal of Physiology, vol. xiv. Most of the other colour reactions of proteids depend on the aromatic radicle they contain (see *Chemical Physiology and Pathology*, p. 123).

is evaporated with an equal quantity of the reagent. Red crystals form, or, if much peptone is present, there will be a red paste. The reaction takes place with 1 part of hydrochloric acid in 10,000. The organic acids do not give the reaction.

(b) Tropæolin test. Drops of a saturated solution of tropæolin OO in 94 per cent. methylated spirit are allowed to dry on a porcelain slab at 40° C. A drop of the fluid to be tested is placed on the tropæolin drop, still at 40° C.; and if hydrochloric acid is present, a violet spot is left when the fluid has evaporated. A drop of 0.006 per cent. hydrochloric acid leaves a distinct mark.

## LESSON XIX

### SECRETION OF SALIVA. ZYMOGEN GRANULES. BLOOD CRYSTALS

**1. Demonstration.**—In a dog which has been anaesthetised the submaxillary gland with its vessels, nerves, and duct has been exposed.<sup>1</sup> A cannula is inserted into the duct. Stimulation of the chorda tympani leads to (*a*) vaso-dilatation ; (*b*) secretion of saliva. Stimulation of the cervical sympathetic leads to (*a*) vaso-constriction ; (*b*) a scanty secretion of very viscid saliva.

After the administration of atropine, the effects of stimulating the nerves on the vessels is still seen, but there is no secretion of saliva.

**2.** Examine microscopically, mounting in aqueous humour or serum (or in glycerine after treatment with osmic acid vapour) small pieces of the pancreas, parotid and submaxillary glands in a normal guinea-pig, and also in one in which profuse secretion has been produced by the administration of pilocarpine.

Note that zymogen granules are abundant in the former, and scarce in the latter, being situated chiefly at the free border of the cells.

**3.** The guinea-pigs in the above experiments should have been killed by bleeding ; collect the blood and defibrinate it by whipping. It may then be utilised as follows :—

(*a*) To a drop add a drop of water and cover ; in a short time crystals of oxyhaemoglobin are formed.

(*b*) Mount another drop in Canada balsam. Crystals of oxyhaemoglobin soon form. These preparations keep for a long time.

(*c*) Shake up some of the blood with a sixteenth of its volume of ether ; let it stand for 20-30 minutes, and mount a drop of the laky fluid. Crystals again form.

These three methods are those most frequently used for obtaining oxyhaemoglobin crystals ; but there is a considerable difference in the readiness with which crystallisation occurs in the blood of different animals. The guinea-pig is one in which crystallisation occurs readily. The crystals are, however, of an exceptional form. In most animals they are rhombic prisms. In the guinea-pig they are rhombic tetrahedra. In the squirrel they are hexagonal (see fig. 30, p. 73).

(*d*) Instead of ether in experiment *c*, use amyl nitrite ; on shaking vigorously the liquid changes to a mahogany colour, and crystals also form. These consist of methaemoglobin, and resemble in form those of oxyhaemoglobin.

(See 'Chemical Physiology and Pathology,' on Saliva, pp. 616-629 ; on Zymogens, pp. 634-636, 655 ; on Blood Crystals, pp. 268-274.)

<sup>1</sup> Directions for this dissection will be found in the *Handbook for the Physiological Laboratory*, p. 470.

## LESSON XX

*HÆMOGLOBIN AND ITS DERIVATIVES*

Defibrinated ox-blood suitably diluted may be used in the following experiments as in those described in Lesson IX.

1. Place some in a hæmatoscope (see fig. 33, p. 77) in front of the large spectroscope. Note the position of the two characteristic bands of oxyhæmoglobin, which is replaced by the single band of hæmoglobin after reduction by the addition of Stokes' reagent or ammonium sulphide. By means of a small rectangular prism a comparison spectrum showing the bright sodium line (in the position of the dark line named D in the solar spectrum) may be obtained, and should be focussed with the absorption spectrum.

2. Obtain similar comparison spectra by the use of the microspectroscope. For this purpose a cell containing a small quantity of oxyhæmoglobin solution may be placed on the microscope stage, and a test-tube containing carbonic oxide hæmoglobin in front of the slit in the side of the instrument. Notice that the two bands of carbonic oxide hæmoglobin are very like those of oxyhæmoglobin, but are a little nearer to the violet end of the spectrum.

Carbonic oxide hæmoglobin may be readily prepared by passing a stream of coal gas through the diluted blood. It has a cherry-red colour, and is not reduced by the addition of ammonium sulphide.

3. **Methæmoglobin.**—Add a few drops of ferricyanide of potassium to dilute blood and warm gently. The colour changes. Place the test-tube in front of the small direct vision spectroscope. Note the characteristic band in the red. On dilution other bands appear. Treat with ammonium sulphide, and the band of hæmoglobin appears.

4. **Acid Hæmatin.**—Add a few drops of glacial acetic acid to dilute blood, and examine with the spectroscope. Compare the position of the absorption band in the red with that of methæmoglobin ; that of acid hæmatin is further from the D line.

Take some undiluted blood and add glacial acetic acid as before. Extract this with ether by gently agitating it with that fluid. The ethereal extract should then be poured off and examined spectroscopically. The band in the red is seen, and on further diluting with ether three additional bands make their appearance.

5. **Alkaline Hæmatin.**—Add to diluted blood a small quantity of strong

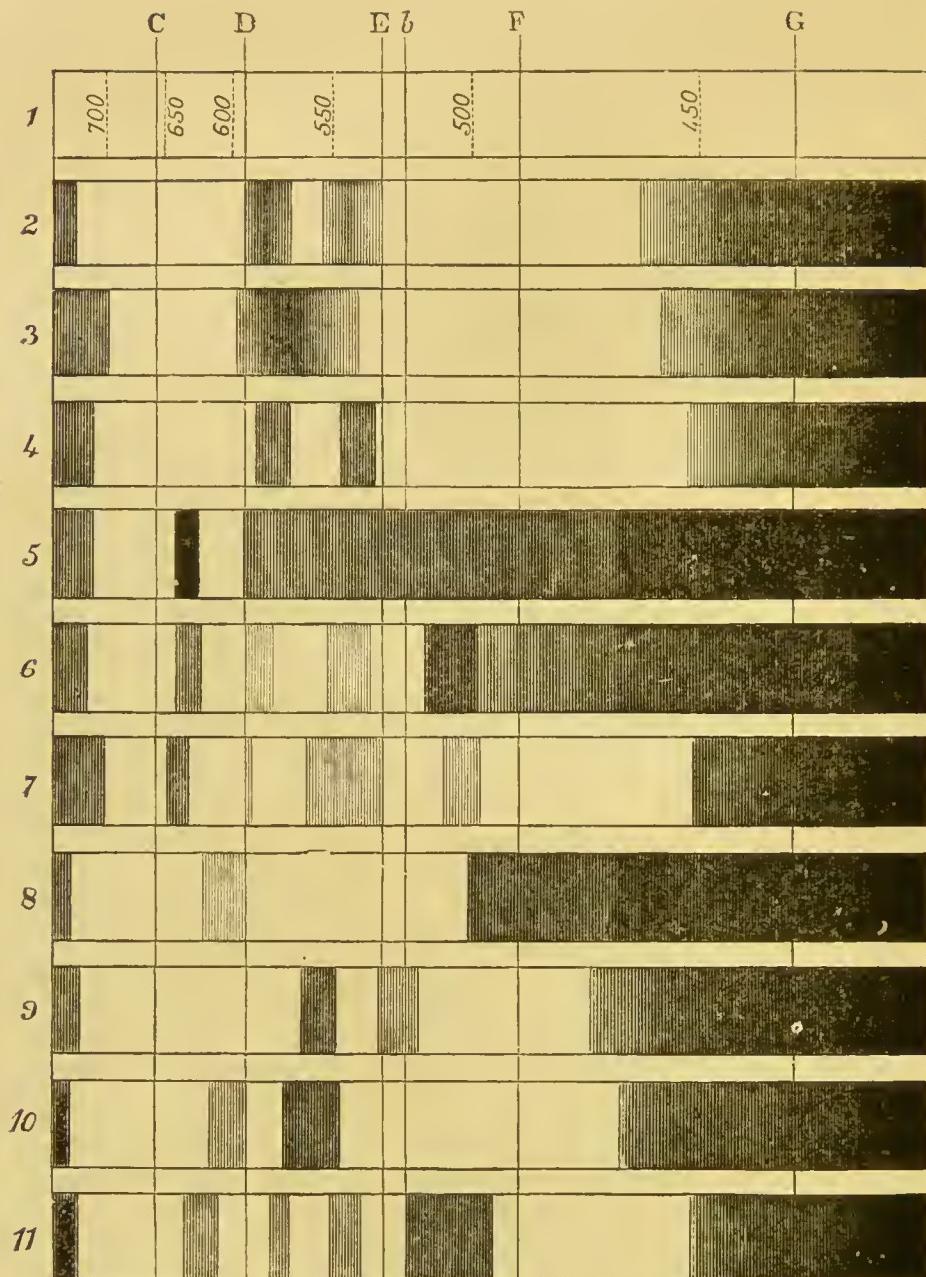


FIG. 59.—1, Solar spectrum. 2, Spectrum of oxyhaemoglobin (0·37 p.e. solution). First band,  $\lambda$  589-564; second band,  $\lambda$  555-517. 3, Spectrum of haemoglobin. Band,  $\lambda$  597-535. 4, Spectrum of CO-haemoglobin. First band,  $\lambda$  583-564; second band,  $\lambda$  517-521. 5, Spectrum of methaemoglobin (concentrated solution). 6, Spectrum of methaemoglobin (dilute solution). First band,  $\lambda$  647-622; second band,  $\lambda$  587-571; third band,  $\lambda$  552-532; fourth band,  $\lambda$  514-490. 7, Spectrum of acid haematin (etheral solution). First band,  $\lambda$  656-615; second band,  $\lambda$  597-577; third band,  $\lambda$  557-529; fourth band,  $\lambda$  517-488. 8, Spectrum of alkaline haematin. Band from  $\lambda$  630-581. 9, Spectrum of haemochromogen (reduced haematin). First band,  $\lambda$  569-512; second band,  $\lambda$  535-504. 10, Spectrum of acid haematoporphyrin. First band,  $\lambda$  697-593; second band,  $\lambda$  585-536. 11, Spectrum of alkaline haematoporphyrin. First band,  $\lambda$  633-612; second band,  $\lambda$  589-564; third band,  $\lambda$  549-529; fourth band,  $\lambda$  518-488. The above measurements (after MacMunn) are in millionths of a millimetre. The liquid was examined in a layer 1 centimetre thick. The edges of ill-defined bands vary a good deal with the concentration of the solutions.

caustic potash and warm. The colour changes, and with the spectroscope a faint shading on the left side of the D line is seen.

6. **Hæmochromogen.**—Add a few drops of ammonium sulphide to a solution of alkaline hæmatin; observe change of colour, and two bands are seen, one between D and E, and the other nearly coinciding with E. The spectrum of alkaline hæmatin reappears for a short time after vigorous shaking with air.

7. **Hæmatoporphyrin.**—To some strong sulphuric acid in a test-tube add a few drops of undiluted blood, and observe the spectrum of acid hæmatoporphyrin (iron-free hæmatin).

8. Map out all the spectra you see on a chart.

The various absorption spectra are depicted in fig. 59.

(See 'Chemical Physiology and Pathology,' p. 274 *et seq.*)

## LESSON XXI

*SERUM*

1. The following methods of precipitating serum-globulin (paraglobulin) should be performed :—

(a) **Panum's Method.**—Dilute serum with fifteen times its bulk of water. It becomes cloudy owing to partial precipitation of the serum-globulin. Add a few drops of 2-per-cent. acetic acid ; the precipitate becomes more abundant, and it dissolves in excess of the acid. It was formerly called 'serum-casein.'

(b) **Alexander Schmidt's Method.**—Dilute serum with twenty times its bulk of water and pass a stream of carbonic acid through it. A fairly abundant precipitate of serum-globulin falls. Let it settle, and an additional precipitate can be obtained from the decanted liquid by treating it with a trace of acetic acid (the 'serum-casein' mentioned above). Repeat the carbonic acid method without dilution ; no precipitate forms.

(c) **By Dialysis.**—Put some serum in a dialyser with distilled water in the outer vessel. The water is frequently changed. In order to prevent decomposition a few crystals of thymol are added. In a few days the salts have passed out ; the proteids remain behind : of these the serum-albumin is still in solution ; the serum-globulin is precipitated, as it requires a small quantity of salt to hold it in solution.

(d) **By Addition of Salts :**—

(i.) **Schmidt's method.** Saturate some serum with sodium chloride. A precipitate of serum-globulin is produced.

(ii.) **Hammarsten's method.** Use magnesium sulphate instead of sodium chloride. A more abundant precipitate is produced, because this salt is a more perfect precipitant of serum-globulin than sodium chloride. In order to obtain complete saturation with these salts it is necessary to shake the mixture of salt and serum for some hours.<sup>1</sup>

(iii.) **Kauder's method.** Half saturate serum with ammonium sulphate. This is done by adding to the serum an equal volume of saturated solution of ammonium sulphate. This precipitates the globulin. Complete saturation with the salt precipitates the albumin also.

2. **Heat Coagulation.**—Saturate serum with magnesium sulphate and filter off the precipitate ; preserve the filtrate and label it 'B.' Wash the precipitate on the filter with saturated solution of magnesium sulphate until the washings do not give the tests for albumin,<sup>2</sup> then dissolve the precipi-

<sup>1</sup> This may be conveniently done by a shaking machine before the glass meets.

<sup>2</sup> On account of the prolonged nature of these operations they must necessarily be performed by the demonstrator beforehand.

tate by adding distilled water. It readily dissolves owing to the salt adherent to it. The solution is opalescent. Label it 'A.'

Render A faintly acid with a drop of 2-per-cent. acetic acid, and heat in a test-tube. The temperature of the test-tube may be raised by placing it in a flask of water gradually heated over a flame. A thermometer is placed in the test-tube, and should be kept moving so as to ensure that all parts of the liquid are at the same temperature. The quantity of liquid in the test-tube should be just sufficient to cover the bulb of the thermometer. A flocculent precipitate of coagulated serum-globulin separates out at about 75°.

Now take the filtrate B. This contains the serum-albumin. Dilute it with an equal volume of water; render it faintly acid as before, testing the reaction with litmus paper. Heat. A flocculent precipitate ( $\alpha$ ) falls at about 73° C.; filter this off; note that the filtrate is less acid than that from which the precipitate has separated, or it may even be alkaline. If so, make it acid again, and heat; a precipitate falls at 77–79° C. ( $\beta$ ). A third precipitate is similarly obtained at 84–86° C. ( $\gamma$ ). In the serum of the ox, sheep, and horse the  $\beta$  and  $\gamma$  precipitates only occur; in cold-blooded animals only the  $\alpha$  variety.

3. Take a fresh portion of B, and saturate it with sodium sulphate. The serum-albumin is precipitated (completely after prolonged shaking). This is due to the formation of sodo-magnesium sulphate. B was already saturated with magnesium sulphate ( $MgSO_4 + 7H_2O$ ); on adding sodium sulphate this double salt ( $MgSO_4 \cdot Na_2SO_4 + 6H_2O$ ) is formed. Shake some serum with sodium sulphate alone. A small precipitate of globulin is produced. Saturate another portion of the serum with sodo-magnesium sulphate; both globulin and albumin are precipitated.

Of the methods used for precipitating serum-globulin practically only two are used now. These are Hammarsten's and Kauder's. The other methods only precipitate the globulin incompletely. Kauder's method is rapid and efficacious: if the globulin is filtered off, the albumin may be precipitated in the filtrate by complete saturation with the same salt, ammonium sulphate. This method avoids the trouble of using two salts as described under 3. This last method is instructive, but not nearly so quick as Kauder's.

With regard to the separation of serum-albumin into  $\alpha$ ,  $\beta$ , and  $\gamma$  varieties by the use of the method of fractional heat coagulation, it must be mentioned that at present no further difference has been shown to exist between them, and the opinion has been very freely expressed that the results obtained are not trustworthy. I am convinced that the method is a good one, especially as in other cases (see MUSCLE) the proteins so separated can be shown to possess other differences. In the case of serum, however—and the same is true for egg-albumin—the matter must still be considered *sub judice*.

(See 'Chemical Physiology and Pathology,' p. 230 *et seq.*)

## LESSON XXII

*COAGULATION OF BLOOD*

1. Sodium sulphate plasma has been prepared by receiving blood into an equal volume of saturated solution of sodium sulphate. After the corpuscles have been separated by the centrifugal machine (one form of this is represented in the next figure), the supernatant plasma is pipetted off.

Dilute some of this with ten times its bulk of water and divide it into five parts—A, B, C, D, and E.

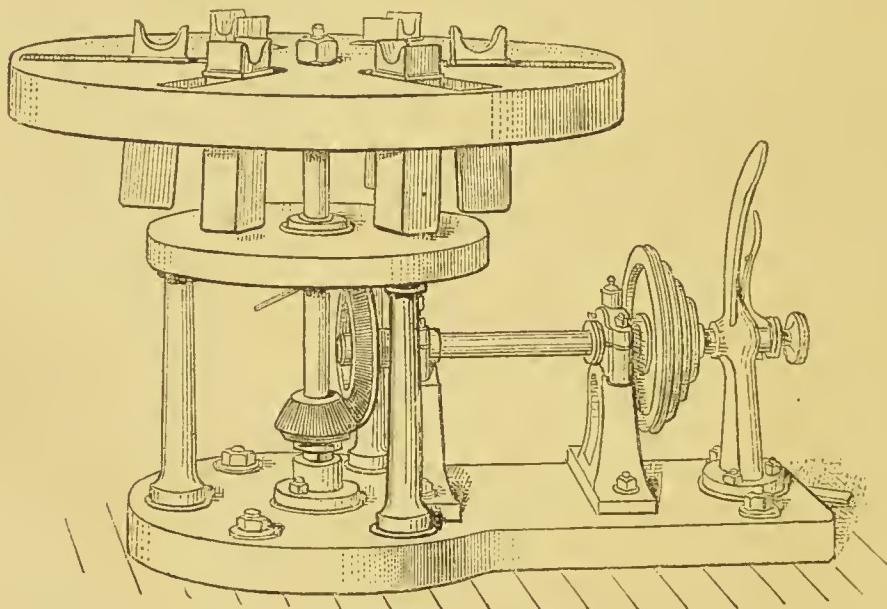


FIG. 60.—Centrifugal machine as made by Rinne, of Basel. Glass vessels containing the substances to be centrifugalised are placed within the six metallic tubes which hang vertically while the disc is at rest; when the machinery is set going they fly out into the horizontal position. A water motor or gas engine may be used to work these instruments. A small but effective hand-centrifuge is made by Watson, Laidlaw & Co., Glasgow.

Leave A at the temperature of the air. It clots slowly or not at all.

Put B into the warm bath at 40° C. It clots more quickly. This shows the influence of temperature in accelerating coagulation. It coagulates because the dilution with water has removed the inhibitory influence of the

strong saline solution on the fibrin ferment which is present as the result of corpuscular disintegration.

To C add a few drops of serum.

To D add a piece of buffy coat.

To E add solution of fibrin ferment dissolved in a weak solution of calcium chloride. Put C, D, and E into the warm bath at 40° C. On account of the presence of abundance of fibrin ferment they clot more quickly than B. The presence of calcium is also essential (see OXALATE PLASMA, Lesson IX.).

3. Heat a portion of the undiluted plasma to 60° C. The fibrinogen is precipitated (coagulated by heat) at 56° C. Filter. Treat the filtrate as in E, diluting it first. No coagulation occurs.

4. Hydrocele Fluid.—This does not clot spontaneously, or only very slowly. Divide it into four parts—A, B, C, and D.

To A add an equal volume of serum.

To B add a few drops of fibrin-ferment solution.

To C add a piece of buffy coat.

Put them into the warm bath, and coagulation takes place in each. The serum or the buffy coat supplies the missing fibrin ferment. The serum does not produce its effect in virtue of the serum-globulin it contains; hydrocele fluid contains both fibrinogen and serum-globulin, as the following experiment shows:—

Take the portion D and half saturate it with sodium chloride by adding to it an equal bulk of saturated solution of sodium chloride. Fibrinogen is precipitated. The precipitate is a small one, and on standing aggregates together, and so becomes more apparent. Filter, and saturate the filtrate with sodium chloride, or, better, magnesium sulphate; serum-globulin is precipitated.

5. Demonstration.—A solution of nucleo-albumin from the thymus has been prepared beforehand by the demonstrator. It may be prepared in one of two ways.

(a) Wooldridge's Method.—The gland is cut up small and extracted with water for 24 hours. Weak acetic acid (0·5 c.c. of the acetic acid of the 'Pharmacopoeia' diluted with twice its volume of water for every 100 c.c. of extract) is then added to the decanted liquid. After some hours the precipitated nucleo-albumin (called tissue-fibrinogen by Wooldridge) falls to the bottom of the vessel. This is collected and dissolved in 1-per-cent. sodium carbonate solution.

(b) The Sodium Chloride Method.—The finely-divided gland is ground up in a mortar with about an equal volume of sodium chloride in a mortar. The resulting viscous mass is poured into excess of distilled water. The nucleo-albumin rises to the surface of the water, where it may be collected and dissolved as before.

A rabbit is anaesthetised, and a cannula inserted into the external jugular vein. The solution is injected into the circulation through this. The animal soon dies from cessation of respiration; the eyeballs

protrude and the pupils are widely dilated. On opening the animal the heart will be found still beating, and its cavities (especially on the right side) distended with clotted blood. The vessels, especially the veins, also are full of clot.

(A general account of blood coagulation is given in Lesson IX. See more fully 'Chemical Physiology and Pathology,' Chap. XV. For recent views the reader is referred to the author's Goulstonian Lectures, *British Medical Journal*, March 1892.)

## LESSON XXIII

*MUSCLE*

1. A rabbit has been killed and its muscles washed free from blood by a stream of salt solution injected through the aorta. The muscles have been quickly removed, chopped up small, and extracted with 5-per-cent. solution of magnesium sulphate. This extract is given out.

2. The extract will probably be faintly acid. The acid is lactic acid. It may be identified by the following reaction :—

A solution of dilute ferric chloride and carbolic acid is made as follows :—

10 c.c. of a 4-per-cent. solution of carbolic acid.

20 c.c. of distilled water.

1 drop of the liquor ferri perchloridi of the British Pharmacopoeia.

On mixing a solution containing only a mere trace (up to 1 part in 10,000) of lactic acid with this violet solution, it is instantly turned yellow. Larger percentages of other acids (for instance, more than 0·2 per cent. of hydrochloric acid) are necessary to decolorise the test solution.

3. The coagulation of muscle is very like that of blood. This may be shown with the salted muscle plasma (the extract given out) as follows :— Dilute some of it with four times its volume of water; divide it into two parts; keep one at 40° C. and the other at the ordinary temperature. Coagulation, that is, formation of a clot of myosin, occurs in both, but earliest in that at 40° C.

4. Add a few drops of 2-per-cent. acetic acid to some of the extract; a stringy precipitate of myosinogen is produced.

5. Remove the clot of myosin from 3; observe it is readily soluble in 10-per-cent. sodium chloride, and also in 0·2-per-cent. hydrochloric acid, forming syntoin.

6. Perform fractional heat coagulation—

(a) With the original extract. Coagula are obtained at 47°, 56°, 63°, 73° C.

(b) With the liquid (salted muscle serum) in 3, after separation of the clot. Coagula are obtained at 63° and 73° C.

(c) With muscle extract which has been saturated with magnesium sulphate and filtered. The globulins are thus separated. Coagulation now occurs at 73° C., but the amount of coagulum is small.

The following table represents these facts concisely :—

Name of protein	Coagulation temperature	Action of $MgSO_4$	Is it globulin or albumin?	Fate
1. Musculin or paramyosinogen }	47° C.	precipitated	globulin	These go to form the muscle clot (myosin)
2. Myosinogen .	56° C.	"	"	These are left in the muscle serum
3. Myo-globulin .	63° C.	"	"	
4. Myo-albumin .	73° C.	not precipitated	albumin	

### 7. Pigments of Muscle :—

- (a) Notice the difference between the red and pale muscles of the rabbit.
- (b) Examine a piece of red muscle (*e.g.* the diaphragm) spectroscopically for oxyhaemoglobin (or it may be more convenient to make an aqueous extract of the muscle and examine that).



FIG. 61.—1, Absorption spectrum of myohæmatin, as seen in muscle rendered transparent by glycerine. 2, Absorption spectrum of modified myohæmatin.

(c) A piece of the pectoral muscle of a pigeon has been soaked in glycerine. Press a small piece between two glass slides and place it in front of the spectroscope. Observe and map out the bands of myohæmatin.

(d) Pieces of the same muscles have been placed in ether for twenty-four hours. The ether dissolves out a yellow lipochrome from the adherent fat. A watery fluid below contains modified myohæmatin. Filter it; compare its spectrum with that of haemochromogen. The myohæmatin bands are rather nearer the violet end of the spectrum (fig. 61) than those of haemochromogen (fig. 59, spectrum 9).

### 8. Creatine :—

(a) Take some of the red fluid described in 7, *d*, and let it evaporate to dryness in an exsiccator over sulphuric acid (fig. 62).

In a day or two crystals of creatine tinged with myohæmatin separate out.

(b) Take an aqueous extract of muscle, like Liebig's extract or beef-tea; add baryta water to precipitate the phosphates, and filter. Remove excess of baryta by a stream of carbonic acid; filter off the barium carbonate and evaporate the filtrate on the water-bath to a thick syrup. Set it aside to

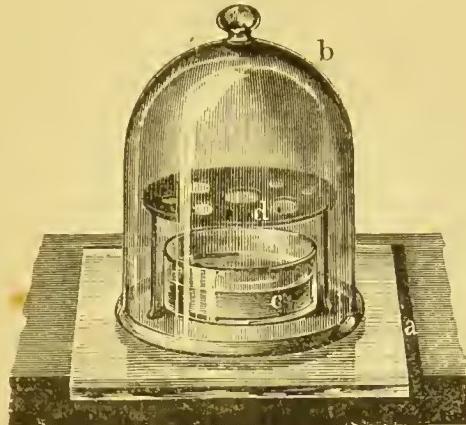


FIG. 62.—An exsiccator. (Gscheidlen.)

cool, and in a few days crystalline deposits of creatine will be found at the bottom of the vessel. These are washed with alcohol and dissolved in hot water. On concentrating the aqueous solution crystals once more separate out, which may be still further purified by recrystallisation.

(See 'Chemical Physiology and Pathology,' Chap. XX. p. 398 *et seq.*)

## LESSON XXIV

### UREA AND CHLORIDES IN URINE

If all the exercises described in the following paragraphs are performed, it may be found convenient to subdivide this lesson into two.

The most important ones are first to estimate the urea by Liebig's method, and then the chlorides, and make the necessary allowance for these; the final result should then be controlled by the use of Dupré's apparatus.

#### ESTIMATION OF UREA

If albumin is present it must be first separated by boiling after acidulation with acetic acid if necessary, and filtering off the flakes of coagulated proteid. The two chief methods of estimating urea are—

- (a) The mercuric nitrate, or Liebig's method.
- (b) The hypobromite, or Hünfner's method.

(a) **Liebig's Method.**—The combination between urea and mercury has the formula  $(\text{CON}_2\text{H}_4)_2\text{Hg}(\text{NO}_3)_2 + 3\text{HgO}$ . It forms a white precipitate, insoluble in water and weak alkaline solutions. It is therefore necessary to prepare a standard solution of mercuric nitrate, and to have an indicator by which to detect the point when all the urea has entered into combination with the mercury, and the latter slightly predominates. This indicator is sodium carbonate, which gives a yellow colour with the excess of mercury, owing to the formation of hydrated mercuric oxide.

Theoretically, 100 parts of urea should require 720 parts of mercuric oxide; but practically 772 of the latter are necessary to remove all the urea, and at the same time show the yellow colour with alkali; consequently the solution of mercuric nitrate must be of empirical strength in order to give accurate results.

The following solutions must be prepared:—

i. Standard mercuric nitrate solution. Dissolve 77.2 grammes of red oxide of mercury (weighed after it has been dried over a water-bath), or 71.5 gr. of the metal itself, in dilute nitric acid. Expel excess of acid by evaporating the liquid to a syrupy consistency. Make up to 1000 c.c. with distilled water, adding the water gradually. This solution is of such a strength that 19 c.c. will precipitate 10 c.c. of a 2-per-cent. urea solution. Add 52.6 c.c. of water to the litre of the mercuric nitrate solution and shake well; then 20 c.c. (instead of 19) = 10 c.c. 2-per-cent. urea solution, i.e. 1 c.c. = .01 urea.

ii. Baryta mixture. This is a mixture of two volumes of solution of barium hydrate with one of solution of barium nitrate, both saturated in the cold.

*Analysis.*—Take 40 c.c. urine. Add to this 20 c.c. baryta mixture and filter off the precipitate of barium salts (phosphates and sulphates). Take 15 c.c. of the filtrate (this corresponds to 10 c.c. of urine) in a beaker. Run into it the mercuric nitrate solution from a burette, until on mixing a drop of the mixture with a drop of a saturated solution of sodium carbonate on a white tile a pale lemon colour appears. Then read the amount used from the burette, and calculate thence the percentage of urea.

*Corrections.*—This method only approaches accuracy when the quantity of urea present is about 2 per cent., which is about the normal percentage of urea in urine. The chlorine in the urine must also be estimated, and the quantity of urea indicated reduced by the subtraction of 1 grammie of urea for every 1·3 grammie of sodium chloride found. If the urine contains less than 2 per cent. of urea, 0·1 c.c. of mercuric nitrate solution must be deducted for every 4 c.c. used; if more than 2 per cent. of urea, a second titration must be performed with the urine diluted with half as much water as has been needed of the mercurial solution above 20 c.c. Suppose, then, 28 c.c. have been used in the first titration, the excess is 8 c.c.; therefore 4 c.c. of water must be added to the urine before the second titration is made. When ammonium carbonate is present, first estimate the urea in one portion of urine, and the ammonia by titration with normal sulphuric acid in another; 0·017 grammie of ammonia = 0·030 of urea. The equivalent of ammonia must be added to the urea found in the first portion of urine.

For modifications of this method which have been proposed see 'Chemical Physiology and Pathology,' p. 811.

*b. The Hypobromite Method.*—This is a far more accurate and easier method.

The method consists in decomposing urea into water, carbonic acid, and nitrogen by means of an alkaline solution of hypobromite of soda; the carbonic acid combines with the soda, and the nitrogen which is evolved is measured, and the quantity of urea therefrom calculated. There are many kinds of apparatus for performing this operation, but the best yet devised are those of Dupré and Gerrard.

The apparatus and reagents one requires for the determination are as follows:—

- i. A Dupré's apparatus<sup>1</sup> or a Gerrard's apparatus.<sup>2</sup>
- ii. A 5 cubic centimetre pipette.
- iii. A strong glass cylinder with a well-fitting glass stopper.
- iv. A 40-per-cent. solution of caustic soda.
- v. Tubes containing 2 and 4 c.c. of bromine.

The two last-named reagents are required for the making of the hypobromite solution, which spoils by keeping (bromato of soda being formed). It should therefore be prepared fresh before every determination.

<sup>1</sup> How & Co., Farringdon Street.   <sup>2</sup> Gibbs, Cuxson & Co., Wednesbury.

The hypobromite solution is made by introducing 23 c.c. of the soda solution into the glass cylinder, then gently dropping in a tube containing 2 c.c. of bromine. The tube is then broken by shaking the cylinder, which is stoppered; the bromine escapes and combines with the soda. This method prevents any inconvenience arising from fumes of bromine. The quantity of solution so prepared is sufficient for one estimation. This method is, as Dupré points out, one of the most valuable points about his

method; the solution can be made with perfect safety by the bedside.

*Method 1 (Dupré).*—Measure 5 c.c. of urine, and introduce it into the test-tube attached to the caoutchouc stopper seen on the upper left-hand side of fig. 63; this will be found simpler to use than the pipette (*e f*) figured below.

Measure 25 c.c. of hypobromite of soda solution, and introduce it into the bottle, *c*.

Close the bottle carefully with the stopper just mentioned, taking care to upset none of the urine in the test-tube attached to it. This stopper is perforated by a glass tube, which is connected by india-rubber tubing to the tube, *a*, by a T-piece. Open the pinch-cock, *d*, and lower the tube, *a*, until the surface of the water, with which the outer cylinder is filled, is at the zero point of the graduation.

Close the pinch-cock, *d*, and raise *a* to ascertain if the apparatus is air-tight; then lower it again. Tilt *c* so as to upset the urine, and shake well for a minute or so.

Immerse *c* in a large beaker containing water of the same temperature as that in the cylinder. After two or three minutes raise the measuring-tube, *a*, until the surfaces of the liquid inside and outside coincide.

Read off the quantity of nitrogen that results from the decomposition of the 5 c.c. of urine by means of the graduations on *a*.

Some of the tubes of Dupré's apparatus are graduated in divisions corresponding to percentages of urea.

The total quantity of urine passed in the twenty-four hours being measured, the total amount of urea excreted in the day can be calculated. If the nitrogen is measured in c.c., 35·4 c.c. of nitrogen corresponds to 0·1 grammes of urea.

*Reactions and Corrections.*—The reaction by which urea is decomposed in this proceeding may be denoted by the following formula:—

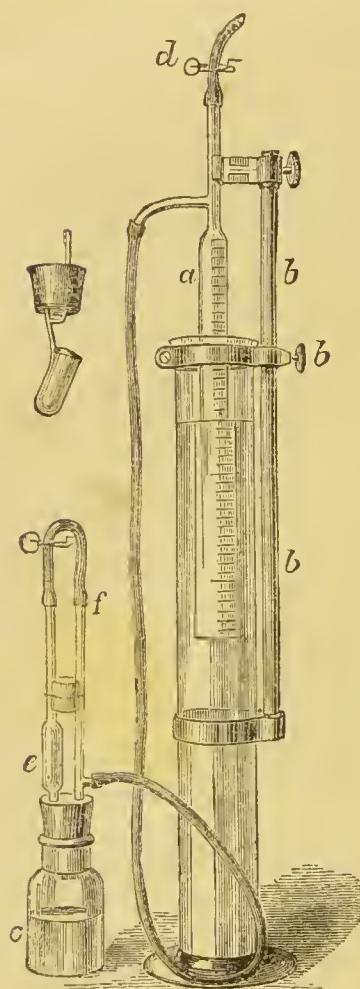


FIG. 63.—Dupré's urea apparatus.

From 1 grammie of urea 0·46 grammie of nitrogen = 372·7 e.e. are obtained.

In practice, however, it is found that only 354·3 e.e. are obtained,<sup>1</sup> except in diabetic urine, in which the urea yields nearly the normal amount of nitrogen. Moreover, urine contains small quantities of creatinine and urates, which yield some of their nitrogen when acted on by sodium hypobromite. When great exactitude is required these must be removed—creatinine by an alcohol solution of zinc chloride, and the urates by acetate of lead followed by sodium phosphate (Yvon).

5 e.e. of a 2-per-cent. solution of urea in urine yield 35·4 e.e. of nitrogen. This quantity is taken as representing 2 per cent. of urea, and serves as a basis for the graduations of the tubes which are marked in percentages.

When very great exactitude is required, the quantity of nitrogen must be measured in cubic centimetres, and the volume obtained corrected for temperature, pressure, and tension of aqueous vapour by the formula below.<sup>2</sup>

*Method 2 (Gerrard).*—In this method the hypobromite solution is prepared as before, and introduced into the bottle, *a* (fig. 64). A stout test-tube containing 5 e.e. of urine is carefully lowered by forceps into this.

By means of the short tube, *c*, the long graduated one is filled with water up to the zero mark: *a* is now connected to this latter tube by india-rubber tubing, as in Dupré's apparatus. The urine and hypobromite are mixed by tilting the bottle, *a*; the nitrogen comes off, and is measured in percentages of urea by the graduations on the tube, *U*. After waiting ten minutes to allow the temperature of the apparatus and the contained gas to reach that of the atmosphere, the water in the two tubes is brought to the same level by lowering the tube, *c*; the reading is then made, and corrected, if necessary, for temperature, pressure, and tension of aqueous vapour as before.

<sup>1</sup> The cause of this loss of nitrogen has been investigated by Luther, *Zeit. physiol. Chem.* xiii. p. 500. He finds part is combined as a nitrate, and part in an unknown organic compound which gives off ammonia when distilled with alkali.

<sup>2</sup>  $V' = \text{correct volume}; V = \text{vol. observed}; B = \text{barometric pressure corrected for temperature}; t = \text{temp. in degrees Centigrade}; T = \text{tension of aqueous vapour in millimetres of mercury at } t^\circ \text{ (see table, p. 7). Then}$

$$V' = \frac{V \times (B - T)}{760 \times (1 + 0.003665t)}.$$

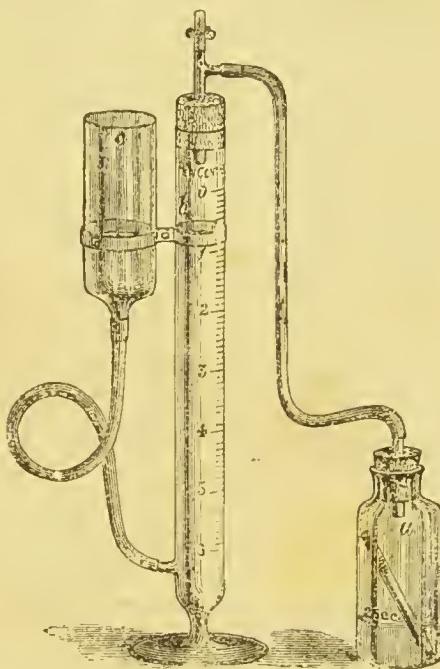


FIG. 64.—Gerrard's urea apparatus.

### ESTIMATION OF CHLORIDES

The chlorides in the urine consist of those of sodium and potassium, the latter only in small quantities.

The method adopted for the determination of the total chlorides consists in their precipitation by a standard solution of silver nitrate or mercuric nitrate.

(a) *Mohr's Method*.—Precipitation by silver nitrate.

The following solutions must be prepared :—

(a) Standard silver nitrate solution. Dissolve 29·075 grammes of fused nitrate of silver in a litre (1000 c.c.) of distilled water ; 1 c.c. = 0·01 gramme of sodium chloride.

(b) Saturated solution of neutral potassium chromate.

*Analysis*.—Take 10 c.c. of urine ; dilute with 100 c.c. of distilled water.

Add to this a few drops of the potassium chromate solution.

Drop into this mixture from a burette the standard silver nitrate solution ; the chlorine combines with the silver to form silver chloride, a white precipitate. When all the chlorides are so precipitated, silver chromate (red in colour) goes down, but not while any chloride remains in solution. The silver nitrate must therefore be added until the precipitate has a pink tinge.

Read off the quantity of standard solution used, and calculate therefrom the quantity of sodium chloride in the 10 c.c. of urine taken, and thence the percentage.

*Sources of Error and Corrections*.—A high-coloured urine may give rise to difficulty in seeing the pink tinge of the chromate of silver : this is overcome by diluting the urine more than stated in the preceding paragraph.

1 c.c. should always be subtracted from the total number of c.c. of the silver nitrate solution used, as the urine contains small quantities of certain compounds more easily precipitable than the chromate.

(b) To obviate such sources of error the following modification of the test, as described by Sutton,<sup>1</sup> may be used :—10 c.c. of urine are measured into a thin porcelain capsule and 1 gramme of pure ammonium nitrate added ; the whole is then evaporated to dryness, and gradually heated over a small spirit lamp to low redness till all vapours are dissipated and the residue becomes white. It is then dissolved in a small quantity of water, and the carbonates produced by the combustion of the organic matter neutralised by dilute acetic acid ; a few grains of pure calcium carbonate to remove all free acid are then added, and one or two drops of potassium chromate. The mixture is then titrated with decinormal silver solution (16·966 gr. of silver nitrate per litre) until the end reaction, a pink colour, appears. Each c.c. of silver solution represents 0·005837 gr. of salt ; consequently, if 12·5 c.c. have been used, the weight of salt in the 10 c.c. of urine is 0·07296 gr., or 0·7296 per cent. If 5·9 c.c. of urine are taken for titration, the number of c.c. of silver solution used will represent the number of parts of salt per 1000 parts of urine.

<sup>1</sup> *Volumetric Analysis*, p. 309.

(c) *Liebig's Method.*—Precipitation by mercuric nitrate.

The following solutions must be first prepared :—

i. Standard mercuric nitrate solution :—Dissolve 20 grammes of pure mercury in boiling nitric acid ; then dilute to nearly a litre. To dilute this to the right strength, preliminary experiments must be performed with a standard solution of pure sodium chloride, 20 grammes to the litre. Take 10 c.c. of the standard sodium chloride solution, add to this 2 c.c. of a 4-percent. solution of urea and 5 c.c. of a saturated solution of sodium sulphate. Into this mixture allow the mercuric nitrate solution to flow from a burette, stirring the mixture the while. A precipitate forms, which redissolves on stirring ; add the mercuric nitrate solution till a permanent precipitate (not an opalescence) forms ; the reaction is then complete. The strength of the mercurial solution is thus determined, and it is then diluted so that 20 c.c. = 0·2 gramme of sodium chloride = 10 c.c. of the standard sodium chloride solution ; 1 c.c., therefore, corresponds to 0·01 gramme of sodium chloride, or 0·006059 gramme of chlorine.

ii. Baryta mixture. This is made by adding two volumes of barium hydrate solution to one of barium nitrate solution, both saturated in the cold.

iii. Dilute nitric acid (1 in 20).

*Analysis.*—Take 40 c.c. of urine.

Add 20 c.c. of baryta mixture. Filter off the precipitate which forms, which consists of sulphate and phosphate of barium.

Take 15 c.c. of the filtrate : this corresponds to 10 c.c. of the original urine.

Render this slightly acid with dilute nitric acid.

Run in the standard mercuric nitrate solution from a burette, stirring the mixture well until a permanent precipitate appears.

Read off the number of c.c. used ; multiply by 0·01. This gives the amount of chlorine as sodium chloride contained in 10 c.c. urine.

*Explanation and Corrections.*—This test depends on the fact that when mercuric nitrate and sodium chloride in solution are mixed, sodium nitrate and mercuric chloride, which are both soluble in water, are formed. It is not till all the chloride in the urine is so decomposed that mercuric nitrate begins to combine with the urea present to form a permanent white precipitate. Hence the necessity of estimating the chlorides when using Liebig's method for the determination of urea.

In order to obtain the exact point at which the precipitate becomes a permanent one, the process must be repeated in another specimen. The advantage of this process is its simplicity : its disadvantage is that the end point is rather obscure.

If the urine used is albuminous, the albumin must be first removed by boiling, after the addition of a few drops of acetic acid, and filtering off the precipitated albumin.

## LESSON XXV

### *ESTIMATION OF PHOSPHATES AND SULPHATES IN URINE*

#### **ESTIMATION OF PHOSPHATES**

The phosphoric acid in the urine is combined with soda, potash, lime, and magnesia.

*(a) Estimation of the total phosphates.*

For this purpose the following reagents are necessary :—

i. A standard solution of uranite nitrate. The uranite nitrate solution contains 35·5 grammes in a litre of water; 1 e.e. corresponds to 0·005 gramme of phosphoric acid ( $P_2O_5$ ).

ii. Acid solution of sodium acetate. Dissolve 100 grammes of sodium acetate in 900 e.e. of water; add to this 100 c.e. of glacial acetic acid.

iii. Solution of potassium ferrocyanide.

*Method.*—Take 50 e.e. of urine. Add 5 e.e. of the acid solution of sodium acetate. Heat the mixture to 80° C.

Run into it while hot the standard uranite nitrate solution from a burette until a drop of the mixture gives a distinct brown colour with a drop of potassium ferrocyanide placed on a porcelain slab. Read off the quantity of solution used and calculate therefrom the percentage amount of phosphoric acid in the urine.

*(b) Estimation of the phosphoric acid combined with lime and magnesia (alkaline earths).*

Take 200 e.c. urine. Render it alkaline with ammonia. Lay the mixture aside for twelve hours. Collect the precipitated earthy phosphates on a filter; wash with dilute ammonia (1 in 3). Wash the precipitate off the filter with water acidified by a few drops of acetic acid. Dissolve with the aid of heat, adding a little more acetic acid if necessary. Add 5 c.c. of the acid solution of sodium acetate. Bring the volume up to 50 e.e., and estimate the phosphates in this volumetrically by the standard uranite nitrate as before. Subtract the phosphoric acid combined with the alkaline earths thus obtained from the total quantity of phosphoric acid, and the difference is the amount of acid combined with the alkalis soda and potash.

*(c)* Instead of uranite nitrate a standard solution of uranite acetate may be used. The directions for the making of these standard solutions will be found in Sutton's 'Volumetric Analysis.' As a rule, it is less troublesome, and not much more expensive, to purchase standard solutions ready-made.

#### **ESTIMATION OF SULPHATES**

The sulphates in the urine are of two kinds: the pre-formed sulphate — viz. those of soda and potash, and the combined or ethereal sulphates.

(a) For the determination of the total amount of sulphuric acid ( $\text{SO}_3$ ) (i.e. pre-formed and combined sulphuric acid together) in the urine, one of two methods is adopted :—

1. Volumetric method.

2. Gravimetric method.

Both methods will be given here; the former is, however, better suited for class experiments.

1. *Volumetric Determination*.—This process consists in adding to a given volume of the urine a standard solution of chloride of barium so long as a precipitate of barium sulphate is formed.

The following solutions are necessary :—

i. Standard barium chloride solution: 30·5 grammes of crystallised chloride of barium in a litre of distilled water; 1 c.c. of this solution corresponds to 0·01 gramme of sulphuric acid ( $\text{SO}_3$ ).

ii. Solution of sulphate of potash: 20 per cent.

iii. Pure hydrochloric acid.

*Method*.—100 c.c. of urine are taken in a flask. This is rendered acid by 5 c.c. of hydrochloric acid, and boiled. The combined sulphates are thus converted into ordinary sulphates, and give a precipitate like them with barium chloride. The chloride of barium solution is allowed to drop into this mixture as long as any precipitate occurs, the mixture being heated before every addition of barium chloride to it. After adding 5 to 8 c.c. of the standard solution, allow the precipitate to settle; pipette off a few drops of the clear, supernatant fluid into a watch-glass; add to it a few drops of the standard barium nitrate solution. If any precipitate occurs, return the whole to the flask and add more barium chloride; again allow the precipitate to settle, and test as before; go on in this way until no more barium sulphate is formed on the addition of barium chloride.

Excess of barium chloride must also be avoided; when only a trace of excess is present a drop of the clear fluid removed from the precipitate gives a cloudiness with a drop of the potassium sulphate solution placed on a glass plate over a black ground. If more than a cloudiness appears, too large a quantity of barium chloride has been added, and the operation must be repeated. From the quantity of barium chloride solution used, the percentage of sulphuric acid in the urine is calculated.

2. *Gravimetric Determination* (i.e. by weight).—This method consists in weighing the precipitate of barium sulphate obtained by adding barium chloride to a known volume of urine; 100 parts of sulphate of barium correspond to 34·33 parts of sulphuric acid ( $\text{SO}_3$ ).

*Method* (Salkowski).—100 c.c. of urine are taken in a beaker. This is boiled with 5 c.c. of hydrochloric acid as before.

Chloride of barium is added till no more precipitate occurs.

The precipitate is collected on a small filter of known ash, and washed with hot distilled water till no more barium chloride occurs in the filtrate—i.e. until the filtrate remains clear after the addition of a few drops of hydric sulphate. Then wash with hot alcohol, and afterwards with ether. Remove

the filter, and place it with its contents in a platinum crucible. Heat to redness. Cool over sulphuric acid in an exsiccator; weigh, and deduct the weight of the crucible and filter ash; the remainder is the weight of barium sulphate formed.

*Error.*—When the experiment is carried out as above there is a slight error from the formation of a small quantity of sulphide of barium. This may be corrected as follows: After the platinum crucible has become cool add a few drops of pure sulphuric acid ( $H_2SO_4$ ). The sulphide is converted into sulphate. Heat again to redness to drive off excess of sulphuric acid.

(b) The following is Salkowski's<sup>1</sup> method of estimating the *combined sulphuric acid*—that is, the amount of  $SO_3$  in ethereal sulphates:—100 c.c. of urine is mixed with 100 c.c. of alkaline barium chloride solution, which is a mixture of two volumes of solution of barium hydrate with one of barium chloride, both saturated in the cold. The mixture is stirred, and after a few minutes filtered; 100 c.c. of the filtrate (= 50 c.c. of urine) are acidified with 10 c.c. of hydrochloric acid, boiled, kept at 100° C. on the water-bath for an hour, and then allowed to stand till the precipitate has completely settled; if possible, it should be left in this way for twenty-four hours. The further treatment of this precipitate (= combined sulphates) is then carried out as in the last case.

*Calculation.*—233 parts of barium sulphate correspond to 98 parts of  $H_2SO_4$ , or 80 parts of  $SO_3$  of 32 parts of S. To calculate the  $H_2SO_4$ , multiply the weight of barium sulphate by  $\frac{98}{233} = 0.4206$ ; to calculate the  $SO_3$  multiply

by  $\frac{80}{233} = 0.34335$ ; to calculate the S multiply by  $\frac{32}{233} = 0.13734$ . This method of calculation applies to the gravimetric estimation both of total sulphates and of combined sulphates.

(c) To obtain the amount of pre-formed sulphuric acid subtract the amount of combined  $SO_3$  from the total amount of  $SO_3$ . The difference is the pre-formed  $SO_3$ .

Example: 100 c.c. of urine gave 0.5 gramme of total barium sulphate. This multiplied by  $\frac{80}{233} = 0.171$  gr. = total  $SO_3$ . Another 100 c.c. of the same urine gave 0.05 gr. of barium sulphate from ethereal sulphates; this multiplied by  $\frac{80}{233} = 0.017$  gr. of combined  $SO_3$ . Total  $SO_3$ —combined  $SO_3$  =  $0.171 - 0.017 = 0.154$  gr. of pre-formed  $SO_3$ .

<sup>1</sup> *Zeit. physiol. Chem.* x. p. 346. This method is a modification of Baumann's original method, *ibid.* i. p. 71.

## LESSON XXVI

## URINE. MISCELLANEOUS EXERCISES

**1. Estimation of Uric Acid.**—Saturate a known volume, say 100 c.c., of urine with crystals of ammonium chloride. Collect the precipitate on a filter, wash it with saturated solution of ammonium chloride, and then dissolve the precipitate in weak alkali (2 per cent. potash).

Neutralise the alkali by the addition of hydrochloric acid. Uric acid is thus precipitated, and is collected on a weighed filter, dried, and weighed.

The above method is that introduced by Hopkins. (For other methods see 'Chemical Physiology and Pathology,' pp. 807-809.)

**2. Estimation of Creatinine.**—The crystalline compound which creatinine forms with zinc chloride is employed in estimating the quantity of creatinine in urine ; 100 parts of the compound correspond to 62·42 of creatinine.

*Method.*—Take 250 c.c. of urine. Add milk of lime and calcic chloride in excess to precipitate the phosphates. Filter, and evaporate the filtrate to a small bulk ; to this add 50 c.c. absolute alcohol, and let the mixture stand for six hours. Then add 10 or 15 drops of an alcoholic solution of zinc chloride ; the crystals form, and after two or three days' standing in a dark place may be collected on a weighed filter.

Wash with 90 per cent. alcohol, dry and weigh, and thence calculate the percentage of creatinine. (For CREATINE and CREATININE see 'Chemical Physiology and Pathology,' pp. 84, 418, 421, 726, 727, 737, 813.)

**3. Urobilin.**—Add neutral and then basic lead acetate to urine until there is no further precipitate. This precipitate carries down most of the pigment with it. Filter ; the filtrate is clear and almost colourless. Extract the urobilin from the precipitate by alcohol acidulated with sulphuric acid ; filter. The filtrate is yellow. Agitate it with chloroform and examine the chloroformic solution (concentrated, if necessary) with the spectroscope. Note the shading in the region of the F line (see 'Chemical Physiology and Pathology,' Chap. XLI. p. 747).

**4. Preparation of Urea from Urine.**—1. Evaporate the urine to a small bulk. Add strong pure nitric acid in excess, keeping the mixture cool during the addition of the acid. Pour off the excess of fluid from the crystals of urea nitrate which are formed ; strain through muslin and press between filter paper. Add to the dry product barium carbonate in large excess. This forms barium nitrate and sets the urea free. Mix thoroughly with sufficient methylated spirit to form a paste. Dry on a water-bath and extract with

alcohol; filter; evaporate the filtrate on the water-bath and set aside. The urea crystallises out, and may be decolourised by animal charcoal and purified by recrystallisation.

2. The following method is well adapted for the preparation of microscopic specimens of urea and urea nitrate: Take 20 c.c. of urine; add 'baryta mixture' (two volumes of barium hydrate solution and one volume of barium nitrate solution, both saturated in the cold) until no further precipitate is produced; filter, evaporate the filtrate to a thick syrup on the water-bath, and extract with alcohol; pour off and filter the aleoholic extract; evaporate it to dryness on the water-bath and take up the residue with water. Place a drop of the aqueous solution on a slide and allow it to crystallise; crystals of urea separate out. Place another drop on another slide and add a drop of nitric acid; crystals of urea nitrate separate out.

5. **Preparation of Pure Uric Acid.**—If one wishes to prepare pure uric acid, the solid urine of a reptile or bird, which consists principally of the acid ammonium salt, should be selected; one has not then to separate any pigment. It is boiled with 10 per cent. caustic soda or ammonia, diluted, and then allowed to stand. The clear fluid is decanted and poured into a large excess of water to which 10 per cent. of hydrochloric acid has been added; after twenty-four hours, crystals of uric acid are deposited. These may be purified by washing, re-solution in soda, and re-precipitation by acid.

## APPENDIX

---

**Gowers's Hæmacytometer.**—The enumeration of the blood corpuscles is readily effected by the hæmacytometer of Gowers. This instrument consists of a glass slide (fig. 65, C), the centre of which is ruled into  $\frac{1}{10}$  millimetre squares and surrounded by a glass rim  $\frac{1}{5}$  millimetre thick. It is provided with measuring pipettes (A and B), a vessel (D) for mixing the blood with a saline solution (sulphate of soda of specific gravity 1015), a glass stirrer (E), and a guarded needle (F).

Nine hundred and ninety-five cubic millimetres of the saline solution are

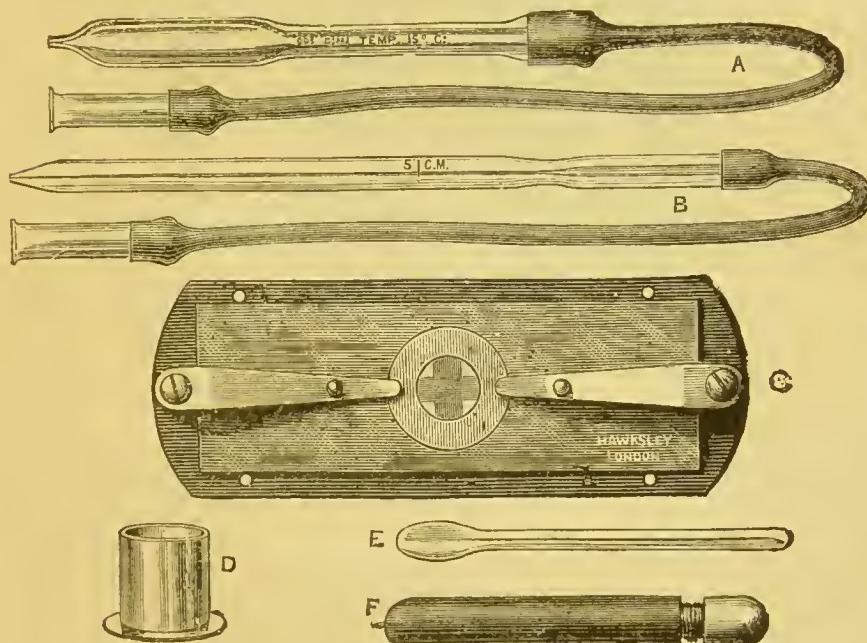


FIG. 65.—Hæmacytometer of Dr. Gowers.

measured out by means of A, and then placed in the mixing jar; 5 cubic millimetres of blood are then drawn from a puncture in the finger by means of the pipette B, and blown into the solution. The two fluids are well mixed by the stirrer, and a small drop of this diluted mixture placed in the centre of the slide C, a cover glass is gently laid on (so as to touch the drop which thus forms a layer  $\frac{1}{5}$  millimetre thick between the slide and cover glass), and pressed down by two brass springs. In a few minutes the

corpuscles have sunk to the bottom of the layer of fluid, and rest on the squares. The number on ten squares is then counted, and this multiplied by 10,000 gives the number in a cubic millimetre of blood. The average number of red corpuscles in each square ought therefore in normal human blood to be 45–50.

**Gowers's Hæmoglobinometer.**—The apparatus consists of two glass tubes, C and D, of the same size. D contains glycerine jelly tinted with carmine to a standard colour—viz. that of normal blood diluted 100 times with distilled water. The finger is pricked and 20 cubic millimetres of blood are measured out by the capillary pipette, B. This is blown out into the tube C, and diluted with distilled water, added drop by drop from the pipette stopper of the bottle, A, until the tint of the diluted blood reaches the standard colour. The tube C is graduated into 100 parts. If the tint of the diluted blood is the same as the standard when the tube is filled up to the graduation 100, the

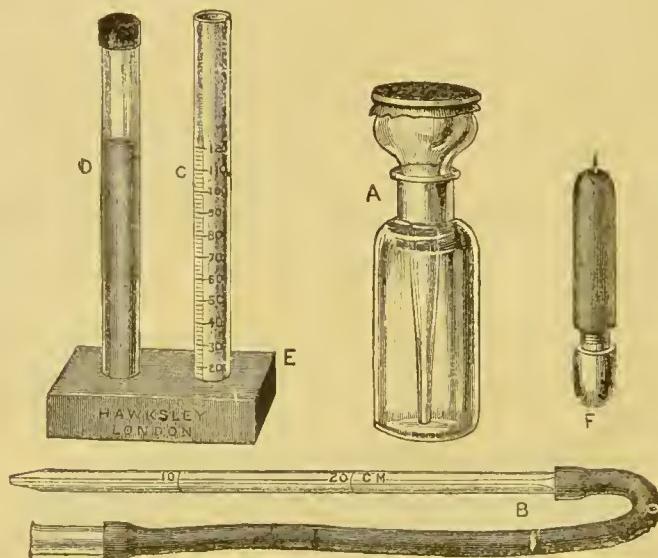


FIG. 66.—Hæmoglobinometer of Dr. Gowers.

quantity of oxyhaemoglobin in the blood is normal. If it has to be diluted more largely, the oxyhaemoglobin is in excess; if to a smaller extent, it is less than normal. If the blood has, for instance, to be diluted up to the graduation 50, the amount of haemoglobin is only half what it ought to be—50 per cent. of the normal—and so for other percentages.

The instrument only yields approximate results, but is extremely useful in clinical observations.

**Von Fleischl's Hæmometer.**—The apparatus (fig. 67) consists of a stand bearing a white reflecting surface (S) and a platform. Under the platform is a slot carrying a glass wedge stained red (K) and moved by a wheel (R). On the platform is a small cylindrical vessel divided vertically into two compartments,  $a$  and  $a'$ .

Fill with a pipette the compartment  $a'$  over the wedge with distilled water. Fill about a quarter of the other compartment ( $a$ ) with distilled water.

Prick the finger and fill the short capillary pipette provided with the instrument with blood. Dissolve this in the water in compartment  $\alpha$ , and fill it up with distilled water.

Having arranged the reflector (S) to throw light vertically through both compartments, look down through them, and move the wedge of glass by the milled head (T) until the colour in the two is identical.

Read off the scale which is so constructed as to give the percentage of haemoglobin.

**Specific Gravity of Blood.**—Of the numerous methods introduced for taking the specific gravity of fresh blood, that of Hammerschlag is the simplest. A drop of blood from the finger is placed in a mixture of chloroform and benzene. If the drop falls, add benzene till it just begins to rise: if the drop

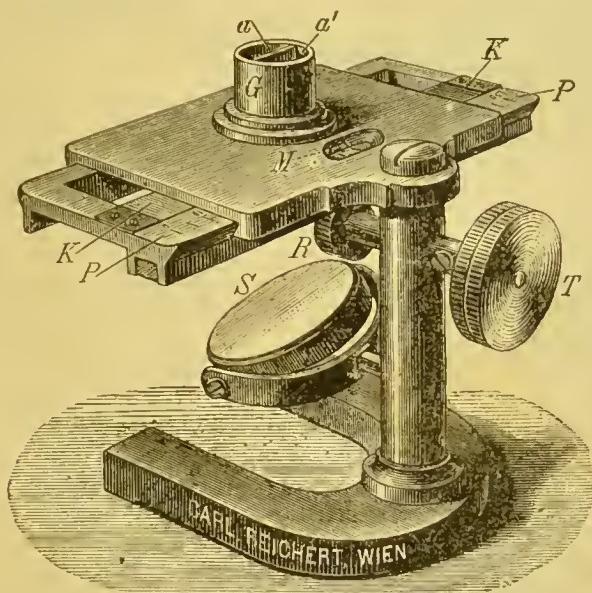


FIG. 67.—Fleischl's haemometer.

rises, add chloroform till it just begins to fall. The fluid will then be of the same specific gravity as the blood. Take the specific gravity of the mixture in the usual way with an accurate hydrometer.

Schmalz's capillary pionometer is a more accurate method.<sup>1</sup>

### POLARIMETERS

**Soleil's Saccharimeter.**—This instrument (see fig. 68) consists of a Nicol's prism,  $d$ , called the polariser: the polarised beam passes next through a quartz plate ( $b$  in fig. 68), 3·75 mm. thick, one half of which ( $d$  in fig. 69) is made of dextrorotatory, the other half ( $g$  in fig. 69) of laevorotatory quartz.

The light then passes through the tube containing the solution in the position of the dotted line in fig. 68, then through a quartz plate cut perpendicularly to its axis ( $q$  in fig. 69), then through an arrangement called

<sup>1</sup> *Deutsch. Arch. f. klin. Med.* xlvi. p. 145.

a compensator (*r* in fig. 69), then through a second nicol called the analyser, and lastly through a telescope (*L* in fig. 69).

The compensator consists of two quartz prisms (*RR*, fig. 69) cut perpendicularly to the axis, but of contrary rotation to the plate just in front of them. These are wedge-shaped and slide over each other, the sharp end of one being over the blunt end of the other. By a screw the wedges may be moved from each other, and this diminishes the thickness of quartz interposed; if moved towards each other the amount of quartz interposed is increased.

The effect of the quartz plate (*d*, *g*) next to the polariser (*c* in fig. 69)

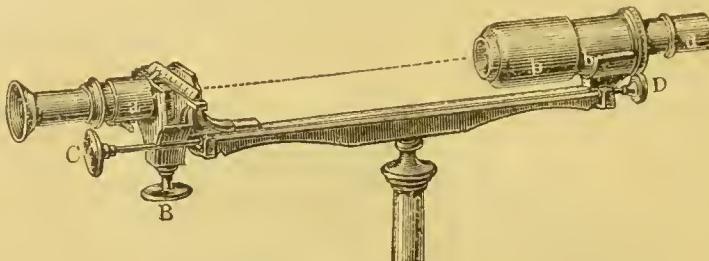


FIG. 68.—Soleil's saccharimeter.

is to give the polarised light a violet tint when the two nicols are parallel to each other. But if the nicols are not parallel, or if the plane of the polarised light has been rotated by a solution in the tube, one half the field will change in colour to the red end, the other to the violet end of the spectrum, because the two halves of the quartz act in the opposite way.

The instrument is first adjusted with the compensator at zero, and the nicols parallel, so that the whole field is of one colour. The tube containing the solution is then interposed; and if the solution is optically inactive the field is still uniformly violet. But if the solution is dextrorotatory the two

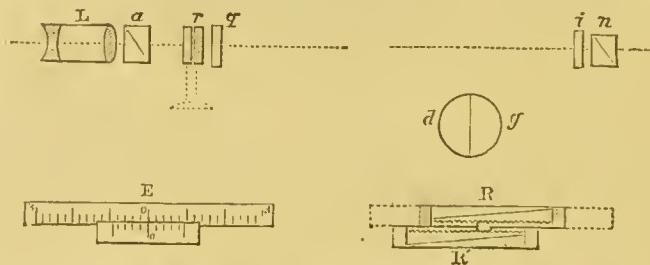


FIG. 69.—Diagram of optical arrangements in Soleil's saccharimeter.

halves will have different tints, a certain thickness of the compensating quartz plate which is laevorotatory must be interposed to make the tint of two halves of the field equal again; the thickness so interposed can be read off in amounts corresponding to degrees of a circle by means of a vernier and scale (*E* in fig. 69) worked by the screw which moves the compensator. If the solution is laevorotatory, the screw must be turned in the opposite direction.

Zeiss's polarimeter is in principle much the same as Soleil's; the chief difference is that the rotation produced by the solution is corrected not by a

quartz compensator but by actually rotating the analyser in the same direction, the amount of rotation being directly read off in degrees of a circle.

Laurent's polarimeter is a more valuable instrument. Instead of using daylight, or the light of a lamp, monochromatic light (a sodium flame produced by volatilising common salt in a colourless gas flame) is employed; the amount of rotation varies for different colours; and observations are recorded as having been taken with light corresponding to the D or sodium line of the spectrum. The essentials of the instrument are, as before, a

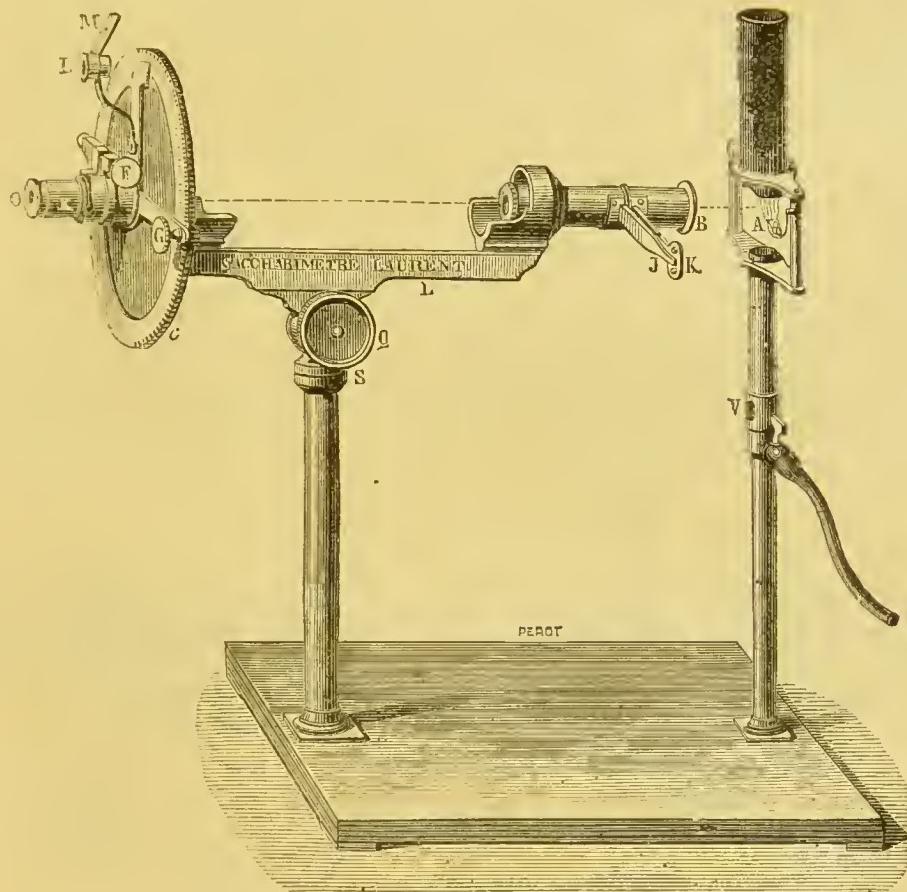


FIG. 70.—Laurent's polarimeter.

polariser, a tube for the solution, and an analyser. The polarised light before passing into the solution traverses a quartz plate, which, however, covers only half the field, and retards the rays passing through it by half a wavelength. In the  $0^\circ$  position the two halves of the field appear equally illuminated; in any other position, or if rotation has been produced by the solution when the nicols have been set at zero, the two halves appear unequally illuminated. This is corrected by means of a rotation of the analyser that can be measured in degrees by a scale attached to it.

Specific rotatory power of any substance is the amount of rotation in

degrees of a circle of the plane of polarised light produced by 1 grammie of the substance dissolved in 1 c.c. of liquid examined in a column one decimetre long.

If  $a$  = rotation observed.

$w$  = weight in grammes of the substance per cubie centimetre.

$l$  = length of tube in decimetres.

$(\alpha)_D$  = specific rotation for light with wave-length corresponding to the D line (sodium flame).

$$\text{Then } (\alpha)_D = \pm \frac{a}{wl}.$$

In this formula + indicates that the substance is dextrorotatory, — that it is laevorotatory.

If, on the other hand,  $(\alpha)_D$  is known, and we wish to find the value of  $w$ , then

$$w = \pm \frac{a}{(\alpha)_D \times l}.$$

### THE SPECTROPOLARIMETER

This instrument is one in which a spectroscope and polarising apparatus are combined for the purpose of determining the concentration of substances which rotate the plane of polarised light. It was invented by E. v. Fleischl for the estimation of diabetic sugar in urine. Its chief advantage is that no difficulty arises of forming a judgment as to the identity of two coloured

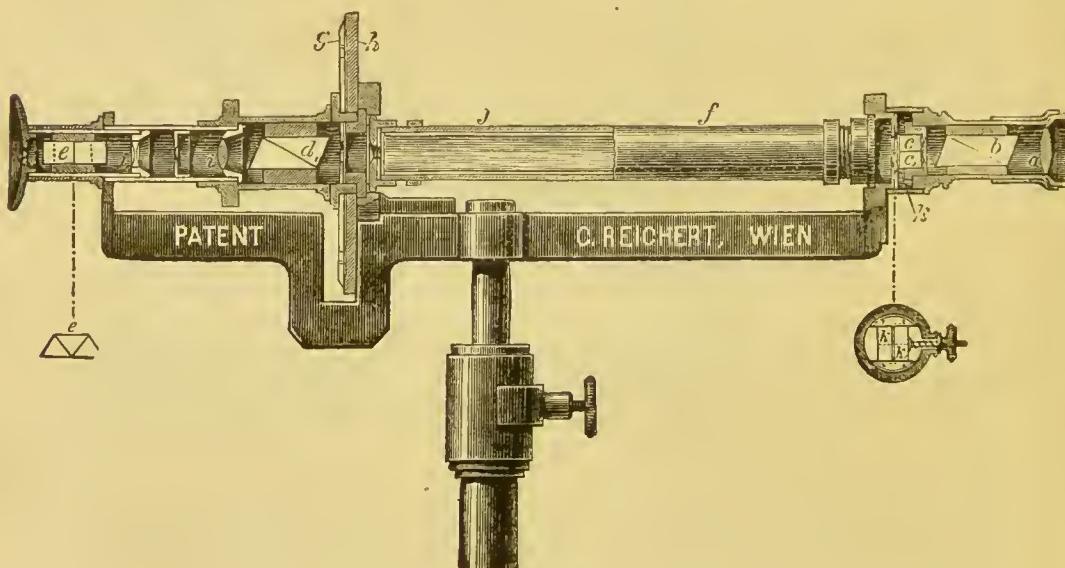


FIG. 71.—Spectropolarimeter of v. Fleischl.

surfaces, as in Soleil's saccharimeter, or of two shades of the same colour, as in Laurent's instrument. The light enters at the right-hand end of the instrument, is polarised by the Nicol's prism  $b$ , and then passes through two quartz plates,  $cc$ , placed horizontally over each other. One of these plates is dextro-, the other laeo-rotatory, and they are of such a thickness (7.75 mm.)

that the green rays between the E and *b* lines of the spectrum are circularly polarised through an angle of 90°, the one set passing off through the upper quartz to the left, the other through the lower to the right. The light then continues through a long tube, *ff*, which contains 15 c.c. of the solution under examination. It then passes through an analysing nicol, *d*, and finally through a direct vision spectroscope, *e*. On looking through the instrument, the tube *ff* being empty, or filled with water or some other optically inert substance, two spectra are seen, one over the other, but each shows a dark band between E and *b* owing to the extinction of these rays by the circular polarisation, produced by the quartz. The analyser can be rotated: a vernier, *g*, is attached to, and moves with it, round a circular disc (seen in section at *h*) graduated in degrees. The two bands in the spectra coincide when the zeros of vernier and scale correspond. If now the tube *f* is filled with an optically active substance like sugar, the bands are shifted, one to the right, the other to the left, according to the direction of rotation of the substance in *f*. The rotation is corrected by rotating the analyser into such a position that the two bands exactly coincide once more as to vertical position. The number of degrees through which it is thus necessary to move the analyser measures the amount of rotation produced by the substance in *f*, and is a measure of the concentration of the solution. The degrees marked on the circular scale are not degrees of a circle, but an arbitrary degree of such a length that each corresponds to 1 per cent. of sugar in the given length of the column of fluid in *ff* (177·2 mm.).

#### PFLÜGER'S MERCURIAL AIR-PUMP

*l* is a large glass bulb filled with mercury; from its lower end a straight glass tube, *m*, about 3 feet long, extends, which is connected by an india-rubber tube, *n*, with a reservoir of mercury, *o*, which can be raised or lowered as required by a simple mechanical arrangement. From the upper end of the bulb, *l*, a vertical tube passes; above the stopcock, *k*, this has a horizontal branch, which can be closed by the stopcock, *f*. The vertical part is continued into the bent tube, which dips under mercury in the trough, *h*. A stopcock, *j*, is placed on the course of this tube. Beyond *f* the horizontal tube leads into a large double glass bulb, *a b*; a mercurial gauge, *e*, and a drying-tube, *d*, filled with pieces of pumice-stone moistened with sulphuric acid being interposed. *a* is called the blood-bulb, and the blood is brought into it by the tube, *c*; the gases, as they come off, cause the blood to froth, and the bulb, *b*, is called the froth-chamber, as it intercepts the froth, preventing it from passing into the rest of the apparatus.

The pump is used in the following way: *l* is filled with mercury, the level in *l* and *o* being the same; *k* is closed; *o* is then lowered, and when it is 30 inches lower than the stopcock *k*, the mercury in *l* falls also, leaving that bulb empty; *j* being closed and *f* open, *k* is then opened, and the air in *a*, *b*, *d*, &c. rushes into the Torricellian vacuum in *l*; *f* is closed and *j* opened; the reservoir, *o*, is raised; the mercury in *l* rises also, pushing the air before it, and it bubbles out into the atmosphere through the mercury (the tube, *h*, is not at this stage in position). When *l* is full of mercury, *k* and *j* are once

more closed and  $o$  is again lowered; when  $l$  is thus rendered once more a vacuum,  $k$  and  $f$  are opened and more of the air remaining in  $a$ ,  $b$ ,  $d$ , &c. rushes into the vacuum;  $f$  is closed,  $j$  is opened, and this air is expelled as before. The process is repeated as often as is necessary to make  $a$ ,  $b$ ,  $d$ , &c.

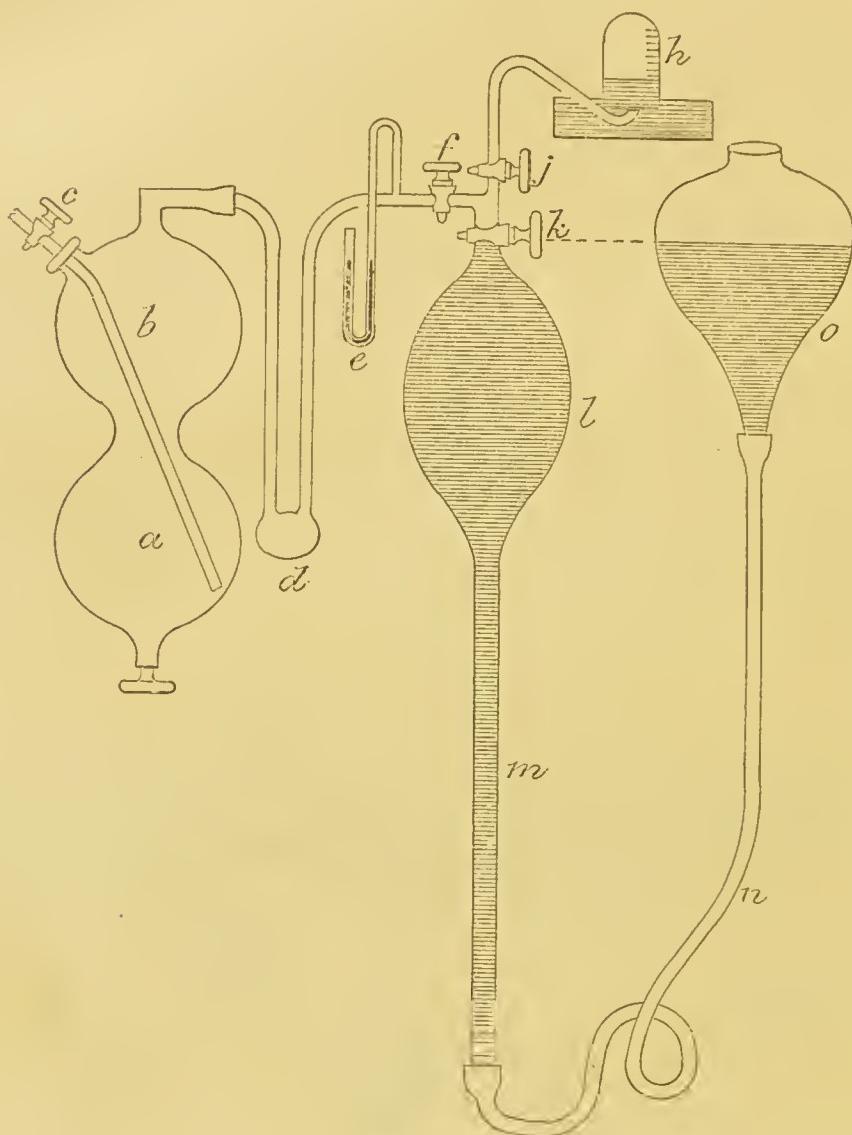


FIG. 72.—Diagram of Pflüger's pump.

as complete a vacuum as indicated by the mercury in the gauge,  $e$ , as is obtainable.

$a$  being now empty and the stopcock,  $f$ , closed, blood is introduced by the tube,  $c$ ; it froths and gives off all its gases, especially if heated to 40–45° C. In the case of serum, acid has to be added to disengage the more firmly combined carbonic acid.<sup>1</sup> The bulb,  $l$ , is once more rendered a vacuum, and

<sup>1</sup> Phosphoric acid is usually employed.

*k* and *f* are opened, *j* being closed. The gas from *a* and *b* rushes into the bulb *l*, being dried as it passes through *d*; *f* is then closed and *j* opened; the reservoir *o* is raised, and as the mercury in *l* rises simultaneously, it pushes the gases into the cylinder, *h*, which is filled with mercury and inverted over the end of the bent tube. This gas can be subsequently analysed. By alternately raising and lowering *o*, and regulating the stopcocks in the manner already described, all the gas from the quantity of blood used can be ultimately expelled into *h*.

A good grease for the stopcocks is a mixture of two parts of vaseline to one of white wax.

Alvergniat's pump has the advantage over Pflüger's of fewer connections, and all of these are surrounded by mercury, which effectually prevents leakage; it has the disadvantage of a rather small bulb in place of *l*, and thus it is more labour to obtain a vacuum.

#### ANALYSIS OF GASES

Waller's modification of Zuntz's more complete apparatus will be found very useful in performing gas analysis, say, of the expired air: a 100 c.c. measuring-tube graduated in tenths of a cubic centimetre between 75 and 100; a filling bulb and two gas pipettes are connected up as in the diagram.

It is first charged with acidulated water up to the zero mark by raising the filling bulb, tap 1 being open. It is then filled with 100 c.c. of expired air, the filling bulb being lowered till the fluid in the tube has fallen to the 100 mark. Tap 1 is now closed. The amount of carbonic acid in the expired air is next ascertained; tap 2 is opened, and the air is expelled into the gas pipette containing strong caustic potash solution by raising the filling bulb until the fluid has risen to the zero mark of the measuring tube. Tap 2 is closed and the air left in the gas pipette for a few minutes, during which the carbonic acid is absorbed by the potash. Tap 2 is then opened and the air drawn back into the measuring tube by lowering the filling bulb. The volume of air (*minus* the carbonic acid) is read, the filling bulb being adjusted so that its contents are at the same level as the fluid in the measuring tube. The amount of oxygen is next ascertained in a precisely similar manner by sending the air into the other gas pipette, which contains sticks of phosphorus

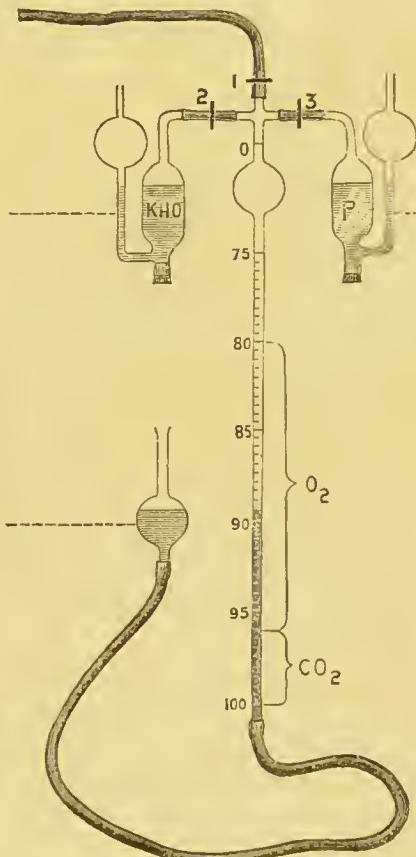


FIG. 73.—Waller's apparatus for gas analysis.

in water, and measuring the loss of volume (due to absorption of oxygen) in the air when drawn back into the tube.

#### KJELDAHL'S METHOD OF ESTIMATING NITROGEN

This simple and accurate method has very largely replaced the older complicated processes.

I take the following account of the method with the modifications proposed by Warington from Sutton's 'Volumetric Analysis.'

From 0·1 to 1 gramme of the dry powdered substance is put into a boiling flask holding about 100-120 c.c. The acid used for the destruction of the organic material is made by mixing 200 c.c. pure oil of vitriol with 50 c.c. Nordhausen oil of vitriol, and 2 grammes of phosphoric acid in sticks; all these must, of course, be free from ammonia: 10-20 c.c. of this mixture is poured over the substance in the flask and heated on wire gauze over a small Bunsen flame. The temperature must be kept below boiling; with prolonged heating the organic matter is gradually destroyed, and the liquid becomes clear and quiet. The nitrogen originally present is thus converted into ammonia, and this may be hastened by adding to the liquid very minute pinches of pure potassium-permanganate. A violent commotion takes place with every addition, but there is no fear of any ammonia being lost. The operation is ended when the mixture becomes permanently greenish (from one to two hours), and moderate heat is continued for a few minutes more. The flask is cooled, some water added, and the contents washed out into a large flask of 700 c.c. capacity with as little water as possible. It is then made alkaline with excess of either pure caustic soda or potash solution (sp. gr. 1·3). A little metallic zinc is added to prevent bumping during the subsequent distillation. The flask is then rapidly closed with a perforated caoutchouc stopper, through which passes an upright tube with two bulbs about an inch in diameter blown upon it: these arrest and carry back any spray of soda from the liquid. The tube above the bulbs is bent over and connected to a condenser, and the delivery end of the condenser leads into a flask containing a measured excess of standard acid. The mixture in the flask is then distilled, the ammonia passes over into the acid. Distillation is continued from forty-five to sixty minutes. The amount of acidity is then determined in the distillate by titration with standard potash or soda, methyl orange being used as the indicator of the end of the reaction. (This gives a pink colour with acid, yellow with alkali.)

*Example.*—Suppose 0·15 gramme of a nitrogenous substance is taken, treated with acid, neutralised, and the ammonia distilled over and received by 100 c.c. of a decinormal solution of hydrochloric acid (= 10 c.c. normal acid). The distillate is then titrated with decinormal soda, and it is found that the neutral point is reached when 60 c.c. of the decinormal soda have been added. The other 40 c.c. must therefore have been neutralised by the ammonia derived from the nitrogenous substance under investigation. This 40 c.c. of decinormal acid = 4 c.c. of normal acid = 4 c.c. of normal ammonia =  $4 \times 0\cdot017 = 0\cdot068$  gramme of ammonia: 0·15 gramme of the substance therefore yields 0·068 gramme of ammonia, and this amount contains 0·056

gramme of nitrogen; 100 grammes of the substance will therefore contain  $\frac{100 \times 0.056}{0.15} = 37.3$  grammes of nitrogen.

The following figure represents the apparatus as modified by Willfarth. In this, oxidation is assisted by adding a small quantity of metallic mercury.

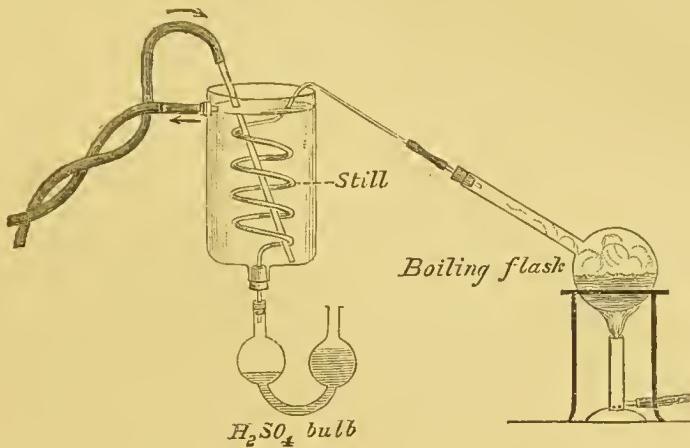


FIG. 74.—Kjeldahl's method. (Waller, after Argutinsky.)

(about  $\frac{1}{10}$  c.c.). To avoid bumping during distillation tale is added instead of zinc. 12 c.c. of strong potassium sulphide solution are also added.

Decinormal sulphuric acid is used as the standard acid, and this is conveniently contained in the bulbs shown in the figure.

#### MICRO-CHEMICAL DETECTION OF PHOSPHORUS

The method of Lilienfeld and Monti is as follows:—The sections of the fresh organ are placed in a strong solution of ammonium molybdate; after about half an hour they are transferred to a 20-per-cent. solution of pyrogallic acid dissolved in ether. They are left here for a few minutes, transferred through spirit to clove oil, and mounted in Canada balsam. The yellow or brown colour, best marked as a rule in the nuclei, indicates the presence of phosphorus. This reaction may be used for distinguishing nucleo-albumin from mucin.

#### MICRO-CHEMICAL DETECTION OF IRON

The tissue is hardened in alcohol, and the sections are transferred to a strong solution of potassium ferrocyanide, acidulated with hydrochloric acid. The bluish-green colour seen indicates the presence of iron.

#### MICRO-CHEMICAL DETECTION OF GLYCOGEN IN LIVER CELLS

A thin piece of liver from a rabbit, fed six hours previously on carrots, is hardened in 90 per cent. alcohol. Sections are cut by the free hand, or after

embedding in paraffin. They are placed in solution of iodine in potassium iodide, then rapidly dehydrated by alcohol, passed into clove oil, and mounted in balsam. The glycogen granules are stained brown. Other sections may be stained with potassium ferrocyanide, acidulated with hydrochloric acid, to show the presence of iron, which is indicated by the blue staining produced.

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